"DEVELOPMENT OF ANALYTICAL METHODS FOR THE SIMULTANEOUS ESTIMATION OF OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE FROM THEIR PHARMACEUTICAL DOSAGE FORMS"

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COIMBATORE - 641 044.

Certificate

This is to certify that the dissertation entitled **"*DEVELOPMENT OF VALIDATED ANALYTICAL METHODS FOR THE ESTIMATION OF TOLTERODINE TARTARATE FROM TABLET DOSAGE FORM*"** was carried out by ***Miss. BINSEY.C.BABU*** in the **Department of Pharmaceutical Analysis,** College of pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore which is affiliated to The Tamil Nadu Dr. M.G.R Medical University, Chennai, under my direct supervision and guidance to my fullest satisfaction.

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CONTENTS

ABBREVIATIONS

UV	-	Ultra violet
VIS	-	Visible
HPTLC	-	High performance thin layer chromatography
HPLC	-	High performance liquid chromatography
RP-HPLC	-	Reverse phase high performance liquid chromatography
λ_{max}	-	Maximum absorbance
nm	-	Nanometer
R_f	-	Retardation factor
HCl	-	Hydrochloric acid
F.C. Reagent	-	Folin ciocalteau Reagent
MBTH Reagent	-	Methyl benzothiazolinone hydrazone hydrochloride Reagent
Conc.	-	Concentrated
µg	-	Microgram
ng	-	Nanogram
M	-	Molar
mM	-	Millimolar
ml	-	Millilitre
µl	-	Microlitre
S.D	-	Standard deviation
R.S.D	-	Relative standard deviation
ICH	-	International conference on harmonization

INTRODUCTION [1-3]

The ability to provide timely, accurate and reliable data is central to the role of analytical chemists and is especially true in the discovery, development and manufacture of pharmaceuticals. Current good manufacturing procedure (cGMP) recommends that every pharmaceutical company have a quality control unit to approve or reject all procedures or specifications that affect the identity, strength, quality and purity of every drug product based on test for drug characteristics such as potency and dissolution.

Some of the key components in quality control which have a great impact on the quality control of the final product are:

1. Scale up
2. Raw material inspection
3. In process inspection
4. Product testing
5. Stability testing

Analytical Techniques

The following analytical techniques have been employed for estimation of different components in formulation.

1. Titrimetric and Gravimetric method.
2. Colorimetric and UV spectrophotometric method.
3. Flame photometry and atomic absorption spectrometry

4. Mass spectroscopy
5. Chromatographic methods which includes;

- Paper Chromatography
- on-exchange chromatography.
- High Performance Thin Layer Chromatography(HPTLC)
- High Performance Liquid Chromatography(HPLC).
- Gas Chromatography (GC).

5. Hybrid Techniques

Combination of the above chromatographic techniques produce 'hybrid' or 'hyphenated' techniques. Several examples are in popular use today and new hybrid techniques are under development. For example

- GC-MS
- LC-MS
- HPLC/ ESI-MS
- LC-DAD
- CE-MS
- CE-UV

ANALYTICAL METHOD VALIDATION

Analytical data are used to screen potential drug candidates, aid in development of drug synthesis, support formulation studies, monitor the stability of bulk pharmaceuticals and formulated products, and test final products for release. The quality of analytical data is a key factor in the success of a drug development program. The process of method

development and validation has a direct impact on the quality of these data.

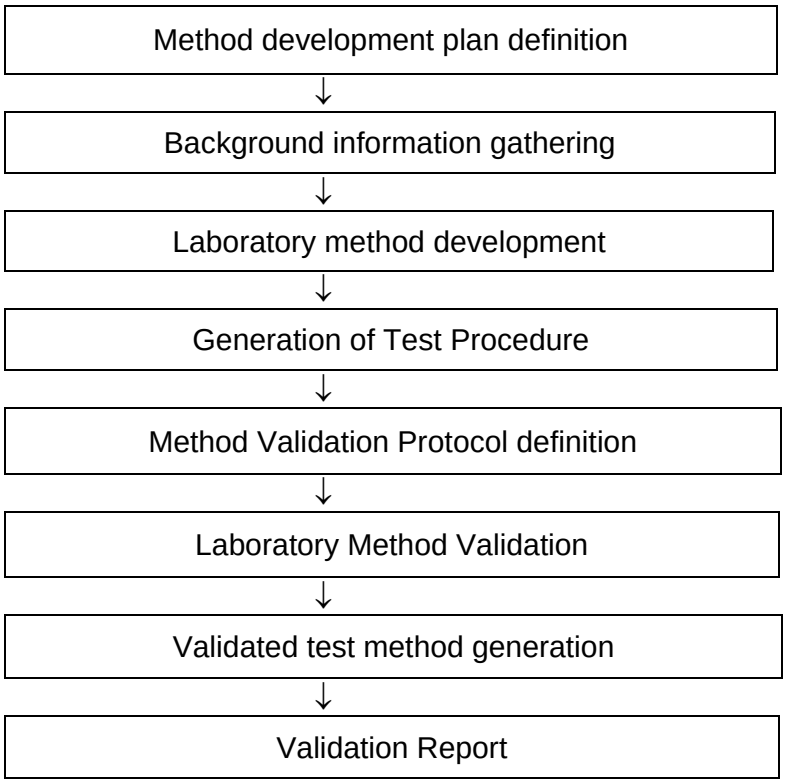
Analytical Method Development is required for:

- Herbal Products
- New process and reactions
- New molecules
- Active Ingredients(Macro analysis)
- Residues(Micro analysis)
- Impurity Profiling
- Component of interest in different matrices.

Analytical Methodology provides to an analyst:

- The required data for a given analytical problem.
- The required sensitivity.
- The required accuracy.
- The required range of analysis.
- The required precision.

Method Validation is the process of proving that an analytical method is acceptable for its intended purpose. Method Validation is a continuous process. The goal is to ensure confidence in the analytical data throughout product development. The steps involved in method development and method validation are:



A well developed method should be easy to validate. A method should be developed with the goal to rapidly test preclinical samples, formulation prototypes, and commercial samples. For validation, the developed method is subjected to the following studies.

1. Specificity

It is the ability of the method to accurately measure the analyte response in the presence of all potential sample components. The response of the analyte in test mixtures containing the analyte and all potential sample components (placebo formulation, synthesis

intermediates, excipients, degradation products, process impurities etc.) is compared with the response of a solution containing only the analyte.

2. Linearity

A linearity study verifies that the sample solutions are in a concentration range where the analyte response is linearly proportional to concentration. Acceptability of linearity data is often judged by examining the correlation coefficient and y-intercept of the linear regression line for the response versus concentration plot.

3. Range

The range of an analytical method is the concentration over which acceptable accuracy, linearity, and precision are obtained. In practice, the range is determined using data from the linearity and accuracy studies.

4. Accuracy

The accuracy of a method is the closeness of the measured value to the true value for the sample. Accuracy is usually determined in four ways:

- It can be assessed by analyzing a sample of known concentration and comparing the measured value to the true value.
- The second approach is to compare test results from the new method with the results from an existing alternate method that is known to be accurate.

- The third approach, which is the most widely used recovery study, is performed by spiking analyte in blank matrices. For assay methods, spiked samples are prepared in triplicate at three levels over a range of 50-150% of the target concentration.
- The fourth approach is the technique of Standard additions, which can also be used to determine recovery of spiked analyte.

5. Precision

The precision of an analytical method is the amount of scatter in the results obtained from multiple analyses of a homogenous sample. It is determined at three levels.

Repeatability

It is obtained when analysis is carried out in one laboratory by one operator using one piece of equipment over relatively short time span for at least 5 or 6 determinations of three different matrices at 2 or 3 different concentrations.

Reproducibility:

It represents the precision obtained between laboratories. The objective is to verify that the method will provide the same results in different laboratories. It is determined by analyzing aliquots from homogenous lots in different laboratories with different analysts with the specified parameters of the method.

6. Limit of Detection (LOD)

The detection limit is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the noise level. **S/N = 3/1**

7. Limit of Quantitation (LOQ)

The quantitation limit is the lowest level of analyte that can be accurately and precisely measured. It is calculated as the analyte concentration that gives **S/N = 10/1**.

8. Robustness

The robustness of a method is its ability to remain unaffected by small changes in parameters such as % organic content and pH of mobile phase, buffer concentration, temperature and injection volume.

LITERATURE REVIEW [4-7]

1. Shinde, D.B., *et al* have reported Spectrophotometric methods for the estimation of Tolterodine tartarate in Bulk and Tablet formulation.

2. Kumar, Y.R., *et al* have reported a validated chiral HPLC method for the enantiomeric separation of Tolterodine tartarate.

3. Vinay, S., *et al* have reported Stability-indicating HPLC determination of Tolterodine tartarate in pharmaceutical dosage form.

4. Perfetto, E.M., *et al* has reported on Treatment of Overactive Bladder- A Model Comparing Extended – Release Formulations of Tolterodine and Oxybutynin.`

AIM AND PLAN OF WORK

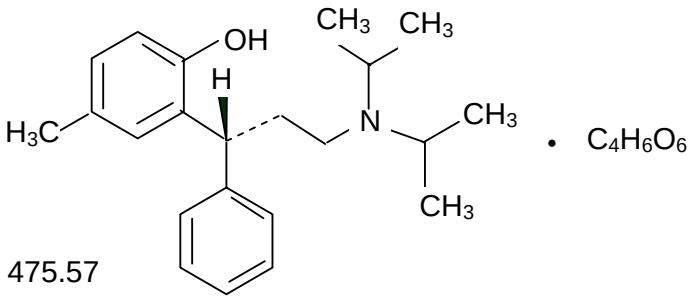
Literature studies revealed that no method has been reported using Difference Spectroscopy, MBTH Reagent, HPTLC and RP-HPLC for the estimation of Tolterodine tartarate in the single dosage form. Hence, the aim and plan of study of this work is as given below:

- Method Development and Validation for the Estimation of Tolterodine tartarate from the Tablet dosage form by Difference Spectroscopy.
- Development of Validated Visible Spectrophotometric Method for the Estimation of Tolterodine tartarate from the Tablet dosage form.
- Method Development and Validation for the Estimation of Tolterodine tartarate from the Tablet dosage form by HPTLC.
- Method Development and Validation for the Estimation of Tolterodine tartarate from the Tablet dosage form by RP-HPLC.

DRUG PROFILE [8-10]

TOLTERODINE TARTARATE

Structure :



Molecular weight : 475.57

Molecular formula : $C_{22}H_{31}NO.\ C_4H_6O_6$

IUPAC Name : 2-[(1R)-3-[Bis (1-methyl ethyl) amino]-1-phenyl propyl]-4-methyl phenol

Description : Tolterodine tartarate occurs as a white crystalline powder.

Solubility : Soluble in water, methanol.

pKa : 9.9

Indications : Used in the treatment of patients with Overactive bladder with symptoms of urge incontinence, urinary frequency. It is a competitive muscarinic receptor antagonist which mediates urinary bladder contraction. It increases residual urine volume and decreases detrussor muscle pressure.

Storage : At 25ºc

Formulation : Roliten(Tolterodine tartarate 2mg), Ranbaxy Laboratories Limited, India.

MATERIALS AND INSTRUMENTS USED

DRUG SAMPLE

Tolterodine tartarate was obtained as a gift sample from Ranbaxy Laboratories Limited, India.

Chemicals and solvents

- Methanol AR grade, HPLC grade (Qualigens Fine Chemicals, Mumbai.)
- Water HPLC grade (Merck Private Limited.)
- MBTH, A.R. (HiMedia Laboratories Pvt.Ltd.)
- Cerric ammonium Sulphate (Loba Chemie)
- Hydrochloric Acid LR grade (sd fine chem limited, Mumbai.)
- Sulphuric Acid (Qualigens Fine Chemicals, Mumbai.)
- Sodium Hydroxide LR grade (sd fine chem limited, Mumbai.)
- Triethylamine AR grade (sd fine chem limited, Mumbai.)
- Glacial acetic acid (sd fine chem, Mumbai.)
- Chloroform (Qualigens Fine Chemicals, Mumbai.)

INSTRUMENTS USED

- Jasco V- 530 UV/VIS Spectrophotometer
- Camag HPTLC System (with Linomat 5 as application device, TLCScanner 3 and winCATS Software)

- Shimadzu HPLC Class LC-10 AT VP system(Photodiode array detector)
- Pall Gelman Sciences, Vacuum pump
- Elico Pvt. Limited, India, pH meter – LI 127
- Shimadzu Digital Electronics Balance – BL220H

INTRODUCTION TO ULTRAVIOLET ABSORPTION SPECTROPHOTOMETRY [11-12]

Ultraviolet Spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (190-380 nm) or 200-400 nm radiation or 2000-4000 Å radiation absorbed by a substance in solution. The wavelengths in the ultraviolet region are usually expressed in angstrom (Å) units or more commonly in millimicron or nanometer; occasionally absorption is reported in wave numbers.

Absorption of light in ultraviolet region of the electromagnetic spectrum occurs when the energy of light matches that required to induce in the molecule an electronic transition and its associated vibrational and rotational transitions.

A compound or drug possess conjugated double bond, absorbs UV radiation at a specific wavelength and this character of drug is specific for a fixed solvent system. The drugs are quantitatively analyzed by ultraviolet analytical method which is governed by Beer Lambert's law, which is represented as

$$A = abc$$

where , a = absorptivity

A =absorbance , b = path length , c = concentration

QUANTITATIVE SPECTROPHOTOMETRIC ASSAY OF MEDICINAL SUBSTANCES

1. Use of a calibration graph

Statistical treatment of the calibration data, facilitated by micro computers or pre programmable calculators, provides a more elegant and accurate determination of the relationship between absorbance and concentration than manually constructed graphs. If the absorbance values and concentrations bear a linear relationship, the regression line $y = \alpha + \beta x$ may be estimated by the **method of least squares.**

$$\alpha = \frac{\left(\sum y\right)\left(\sum x^2\right) - \left(\sum x\right)\left(\sum xy\right)}{N\sum x^2 - \left(\sum x\right)^2}$$

$$\beta = \frac{N\sum xy - \left(\sum x\right)\left(\sum y\right)}{N\sum x^2 - \left(\sum x\right)^2}$$

Where, x = concentration

y = absorbance

N = number of pairs of values

2. Single - point standardization method

The single -point procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The standard and sample solutions are prepared in a similar manner. Ideally, the concentration of the standard solution should

be close to that of the sample solution. The concentration of the substance in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

$$c_{test} = \frac{A_{test} \times c_{std}}{A_{std}}$$

Where c_{test} and c_{std} are the concentrations in the sample and standard solutions respectively, and A_{test} A_{std} are the absorbances of the sample and standard solutions respectively. Since sample and standard solutions are measured under identical conditions, this procedure is the preferred method of assay of substances that obey Beer's Law and for which a reference standard of adequate purity is available

ASSAY OF SUBSTANCES IN MULTICOMPONENT SAMPLES

The various spectroscopic techniques used for multi-component analysis are as follows

- Simultaneous equation method (Vierodt's method)
- Absorbance ratio method
- Geometric correction method
- Absorption factor method (absorption correction method)
- Orthogonal polynomial method
- Difference spectrophotometry
- Derivative spectrophotometry

Simultaneous equation method

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equation (Vierodt's method).

The information required is

a) The absorptivities of X at λ_1 and λ_2 , a_{x1} and $a_{x2,}$ respectively.

b) The absorptivities of Y at λ_1 and λ_2 , a_{y1} and $a_{y2,}$ respectively.

c) The absorbances of the diluted sample at λ_1 and λ_2 , A_1 and $A_{2,}$ respectively.

Let C_x and C_y be the concentration of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at λ_1 and $\lambda_{2,}$ the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At λ_1 $A_1 = a_{x1}bc_x + a_{y1}b_{cy}$

At λ_2 $A_2 = a_{x2}bc_x + a_{y2}b_{cy}$

$$C_x = \frac{A_2\, a_{y1} - A_1\, a_{y2}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

$$C_y = \frac{A_1\, a_{x1} - A_2\, a_{x1}}{a_{x2}\, a_{y1} - a_{x1}\, a_{y2}}$$

Using the above equations, concentration of individual component in a mixture can be determined by simple calculation.

Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that, for a substance which obeys Beer law at all wavelengths, the ratio of absorbances at any two wavelengths is a constant value independent of concentration or path length. Two different dilutions of the same substance which gives the same absorbance ratio is referred to as Q value.

In the quantitative assay of two components in admixture by the absorbance ratio method, absorbance are measured at two wavelengths, one being the λ_{max} of one of the components and the other being a wavelength of equal absorptivity of the two components, i.e. an iso-absorptive point.

Geometric correction method:

This method reduces or eliminates the background irrelevant absorption that may be present in samples of biological origin. This is applied if the irrelevant absorption is linear at the three wavelengths selected.

Consider an absorption spectrum comprising the spectrum of an analyte and that of the background absorption. If the wavelengths λ_1, λ_2 and λ_3 are selected so that the background absorbances B_1, B_2 and B_3 are linear, then the corrected absorbance, D of the drug may be calculated from the three absorbances A_1, A_2 and A_3 of the sample solution at λ_1, λ_2

and λ_3. Vitamin A is assayed by this method.

$$D = \frac{y\,(A_2-A_3)+z(A_2-A_1)}{(y+z)\,(1-r)}$$

Orthogonal polynomial method:

This technique is another mathematical correction procedure which involves more complex calculations than the three-point correction procedure. The basis of the method is that an absorption spectrum may be represented in terms of orthogonal functions as follows:

$$A\,(\lambda) = p_0P_0\,(\lambda) + p_1P_1\,(\lambda) + p_2P_2\,(\lambda)\ldots.\ P_nP_n\,(\lambda)$$

where A denotes the absorbance at wavelength λ belonging to a set of n+1 equally spaced wavelengths at which the orthogonal polynomials,$P_0(\lambda)$, $P_1(\lambda)$, $P_2(\lambda)\ldots.P_n(\lambda)$ are each defined. These polynomials represent a series of fundamental shapes.

The coefficients are proportional to the concentration of the absorbing analyte, and a modified Beer-Lambert's equation may be constructed.

$$p_j = \alpha_j bc$$

p_j = the coefficient of the polynomial

α_j = proportionality constant analogous to absorptivity.

The accuracy of the orthogonal functions procedure depends on the correct choice of the polynomial order and set of the wavelengths. The set of wavelengths is defined by the number of wavelengths, the interval and

the mean wavelength of the set (λ_m). The wavelength interval and λ_m are best obtained from a convoluted absorption curve. This is a plot of the coefficient (p_j) for a specified order of polynomial, a specified number of wavelengths and a specified wavelength interval against the λ_m of the set of wavelengths.

Derivative spectrophotometry

Derivative spectrophotometry involves the conversion of a normal spectrum to its first, second or higher derivative spectrum. The transformations that occur in the derivative spectra are understood by reference to a Gaussian band, which represents an ideal absorption band.

The first derivative (D1) spectrum is a plot of the rate of change of absorbance with wavelength against wavelength or a plot of $dA/d\lambda$. The second derivative (D2) spectrum is a plot of the curvature of the D° spectrum against wavelength or a plot of $d2/d\lambda 2$ vs λ.

These spectral transformations confer two principal advantages on derivative spectrophotometry.

a) Derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of the λ_{max} of the individual bands.

b) Derivative spectrophotometry discriminates in favour of substances of narrow spectral bandwidth against broad bandwidth substances.

INTRODUCTION TO DIFFERENCE SPECTROSCOPY[11-15]

Difference Spectroscopy, provides a sensitive method for detecting small changes in the environment of a chromophore, or it can be used to demonstrate ionization of a chromophore leading to the identification and quantification of various components in a mixture. In difference spectroscopy, absorption spectra of two samples of slightly different composition or physical state are compared.

In difference spectroscopy, a double-beam instrument is used to measure the difference in absorbance between the two samples. Instead of a blank solution, one of the two solutions is placed in the path of the reference beam of the spectrophotometer. The other solution is placed in the usual position in the path of the sample beam. The instrument measures the difference in absorbance between the two samples. Difference spectrophotometry is particularly useful for measuring small differences between two, nearly identical solutions. Difference spectra are particularly useful for determining qualitative differences between two solutions.

The selectivity and accuracy of spectrophotometric analysis of samples containing absorbing interferents may be markedly improved by the technique of difference spectrophotometry. The essential feature of a difference spectrophotometric assay is that the measured value is

the difference absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics.

The criteria for applying difference spectrophotometry to the assay of a substance in the presence of other absorbing substances are that:

- Reproducible changes may be induced in the spectrum of the analyte by the addition of one or more reagents.
- The absorbance of the interfering substances is not altered by the reagents.

The simplest and most commonly employed technique for altering the spectral properties of the analyte is the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. The ultraviolet-visible absorption spectra of many substances containing ionisable functional groups, e.g., phenols, aromatic carboxylic acids and amines are dependent on the pH of the solution. The difference absorption spectrum is generated automatically using a double beam recording spectrophotometer.

The measured value in a quantitative difference spectrophotometric assay is the ΔA at any suitable wavelength measured to the baseline.

$$\Delta A = A_{alk} - A_{acid}$$

where, A_{alk} and A_{acid} are the individual absorbances at wavelength in different medium.

If A_{alk} and A_{acid} are proportional to the concentration of the analyte and pathlength, the ΔA obeys Beer-Lambert law and a modified equation may be derived.

$$\Delta A = \Delta abc$$

where, Δa = difference absorptivity of the substance at the wavelength of measurement.

If one or more other absorbing substances are present in the sample which at the analytical wavelength has identical absorbance (A_x) in the alkaline and acidic solutions, its interference in the spectrophotometric measurement is eliminated.

$$\Delta A = (A_{alk} + A_x) - (A_{acid} - A_x)$$

$$= A_{alk} - A_{acid}$$

The selectivity of the ΔA procedure depends on the correct choice of the pH values to induce the spectral change of the analyte without altering the absorbance of the interfering components of the sample. The use of alkali and analyte to induce the ΔA of the analyte is convenient and satisfactory when the irrelevant absorption arises from pH-insensitive substances.

The selection of two buffers, one at a pH two units greater than, and the other at a pH two unit less than, the pK_a of the analyte, ensure

that the analyte is 99% in the ionized state and that the ∆A is most maximal.

A substance whose spectrum is unaffected by changes of pH may be determined by a difference spectrophotometric procedure if it can be quantitatively converted by means of a suitable reagent to a chemical species that has different spectral properties to its unreacted parent substance.

The ∆A between equimolar solutions of the unreacted substance and its derivative is free of interference if the irrelevant absorption is unaffected by the reagent

Applications

1. Difference spectroscopy is utilized in toxicology laboratories for analysis of dangerous drugs.
2. It is used to study the conformation of globular proteins in solution.
3. The difference spectrum gives the information which has been obtained using solvent perturbation, pH difference, temperature difference and concentration difference etc.

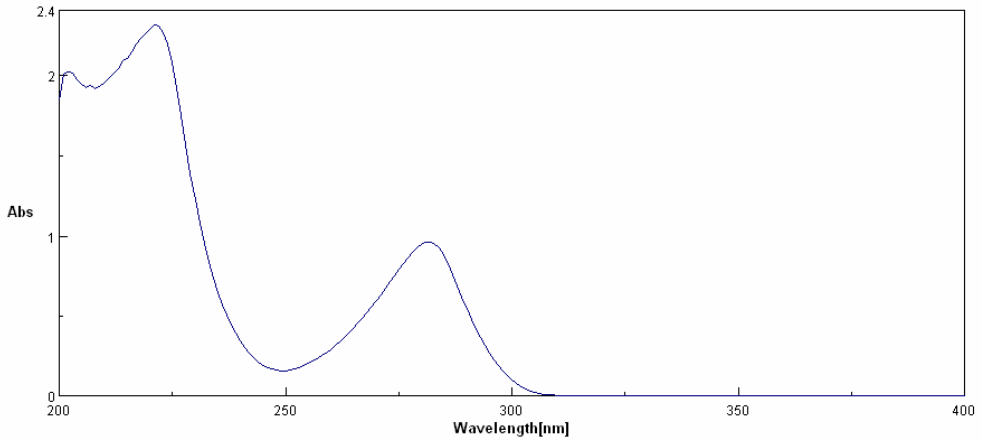
METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TOLTERODINE TARTARATE FROM TABLET DOSAGE FORM BY DIFFERENCE SPECTROSCOPY

METHOD DEVELOPMENT

1. Selection of Solvent and Wavelength

From the literature studies, solubility studies, it was found that Tolterodine tartarate was soluble in water and the drug showed good stability in this solvent. The drug was dissolved in water and the absorbance and spectral pattern was noted. Tolterodine tartarate showed a λ_{max} of 282 nm in water. The absorption spectrum is shown in Fig. 1.

Fig.1 : Absorption spectrum of Tolterodine tartarate in water



2. Preparation of stock solution

10 mg of Tolterodine tartarate was weighed and transferred into a 100 ml standard flask, dissolved in water and made upto volume with the same to get a concentration of 100 µg/ml.

3. Difference Spectroscopy

i) Optimizing the molarity of hydrochloric acid

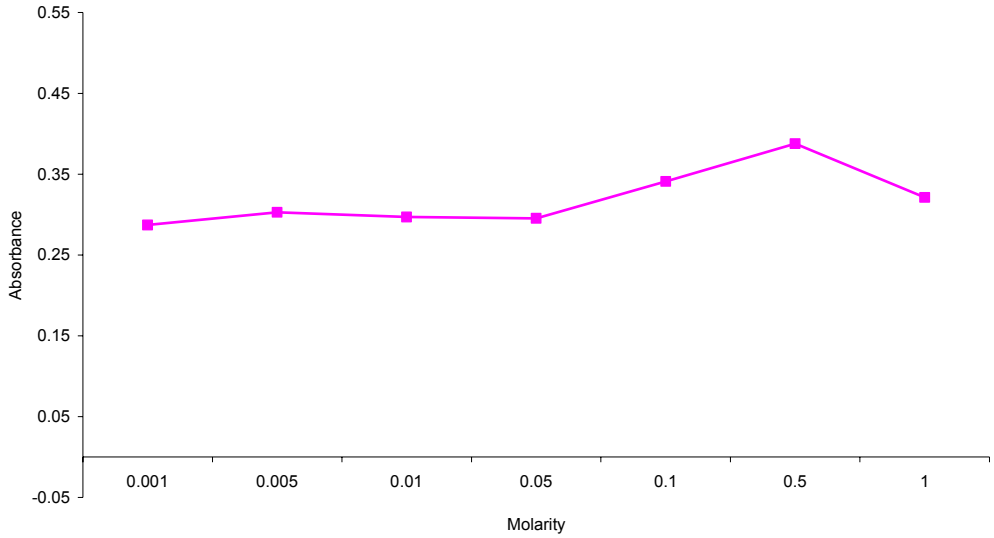
Stock solution of 5.0 ml was pipetted into a series of 10 ml standard flasks .To this, different molarities of hydrochloric acid such as 1M, 0.5M, 0.1M, 0.05M, 0.01M, 0.005M, 0.001M were added and made upto volume with the same to produce solutions of concentration 50 µg/ml. **The absorbance and spectral pattern were noted and the absorbance was found to be the highest for 0.5M HCl. (**Table :1 and Fig. 2)

Table 1: Fixing the Molarity of Hydrochloric acid

Concentration (µg/ml)	Molarity	Absorbance
50	0.001M	0.2870
	0.005M	0.3029
	0.01M	0.2971
	0.05M	0.2954
	0.1M	0.3410
	0.5M*	**0.3877**
	1M	0.3213

* Selected parameter

Fig. 2 : Optimizing the molarity of hydrochloric acid



ii) Optimizing the molarity of sodium hydroxide

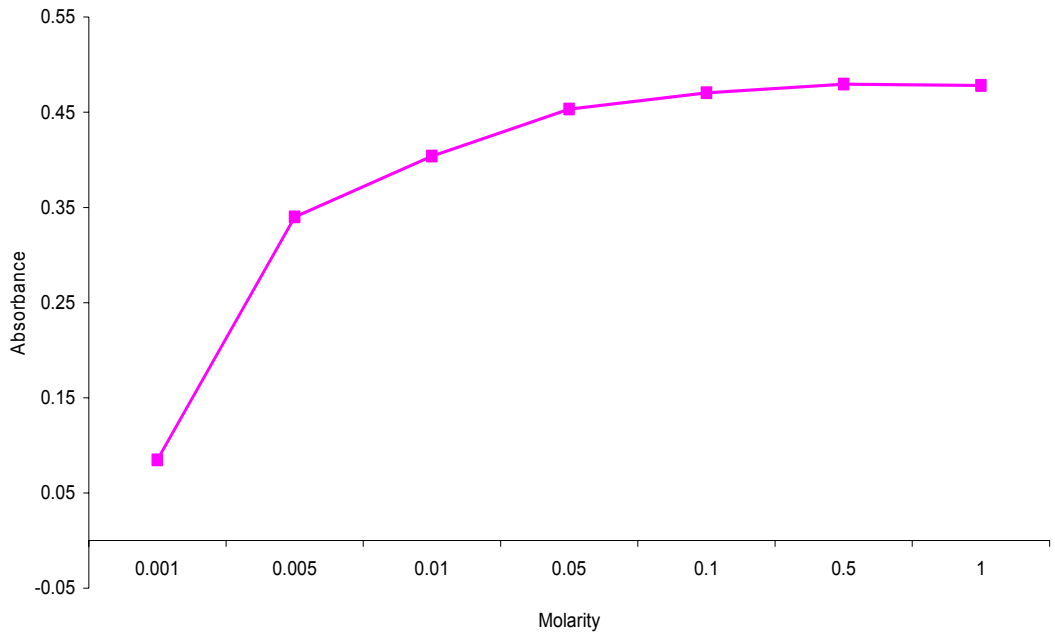
Stock solution of 5.0 ml was pipetted into a series of 10 ml standard flasks. To this, different molarities of Sodium hydroxide such as 1M, 0.5M, 0.1M, 0.05M, 0.01M, 0.005M, 0.001M were added and made upto volume with the same to produce solutions of concentration 50 µg/ml. **The absorbance and spectral pattern were noted and the absorbance was found to be the highest for 0.5M NaOH.** (Table:2 and Fig. 3)

Table 2 : Fixing the Molarity of Sodium hydroxide

Concentration (µg/ml)	Molarity	Absorbance
50	0.001M	0.0848
	0.005M	0.3400
	0.01M	0.4038
	0.05M	0.4532
	0.1M	0.4704
	0.5M*	**0.4795**
	1M	0.4780

* Selected parameter

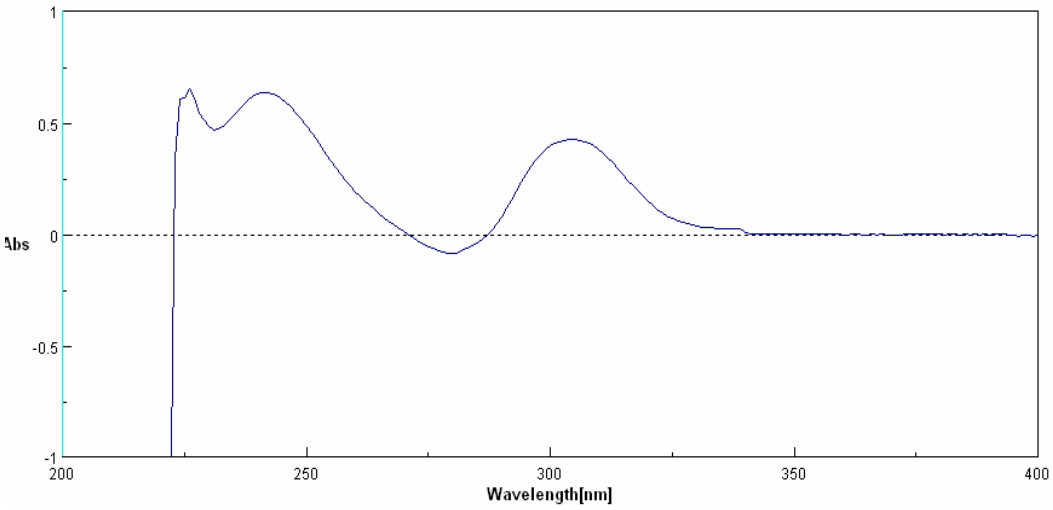
Fig. 3 : Optimizing the molarity of sodium hydroxide



iii) Obtaining the Difference Spectra

The difference spectra of equimolar solutions (0.5 M) of Tolterodine tartarate, an urogenital antispasmodic agent, in both HCl and NaOH were taken and are shown in Fig. 4.

Fig. 4 : Difference spectra



The use of this concentration to induce the ΔA of the analyte was convenient and satisfactory. Hence, this molarity was chosen for the proposed study.

iv) Preparation of Calibration Graph

Aliquots of stock solution (100 µg/ml) of the drug ranging from 1 to 9 ml were transferred to two sets of a series of 10 ml volumetric flasks. One set of the stock solution was diluted with 0.5M HCl to volume and the second set was diluted with 0.5M NaOH to volume. Difference spectrum was recorded by placing same concentration of the acidic and alkaline

solution in the reference and sample cell respectively. The overlay spectra of standards are shown in Fig. 5. Difference of absorbance at two different wavelengths - 280 nm and 305 nm was calculated. (Table : 3). The difference in absorbance (∆A) was plotted versus concentration (10-90 µg/ml), calibration curve was constructed, and the slope, intercept and regression equation was calculated. (Fig. 6)

Table 3 : Difference in absorbance values of Tolterodine tartarate

Concentration (µg/ml)	Absorbance		∆A
	305 nm	280 nm	
10	0.1322	-0.0046	0.1368
20	0.2422	-0.0408	0.2830
30	0.3414	-0.0768	0.4182
40	0.4449	-0.1182	0.5632
50	0.5514	-0.1497	0.7012
60	0.64684	-0.1917	0.8386
70	0.7568	-0.2259	0.9827
80	0.8519	-0.2627	1.1146
90	0.9836	-0.2807	1.2643

Fig. 5 : Overlay spectra of standards

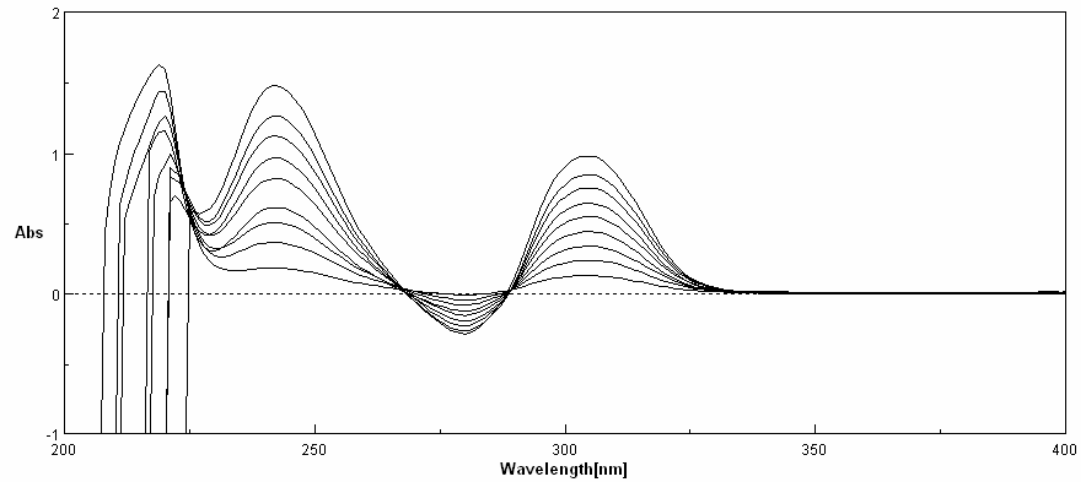
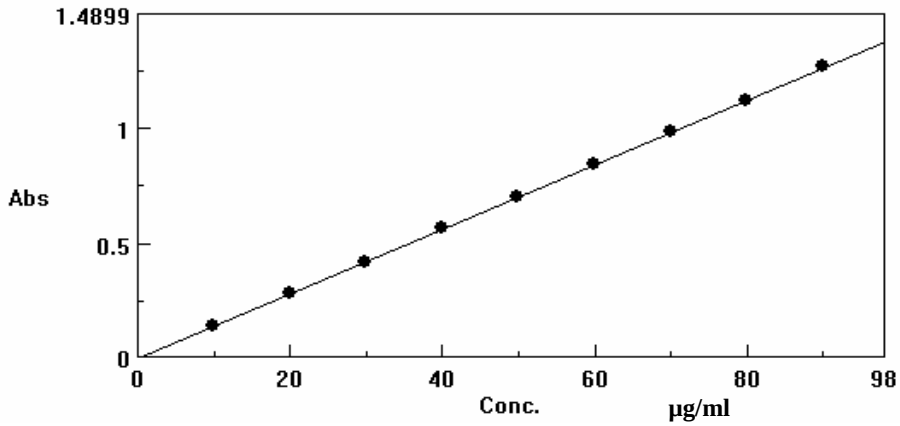


Fig. 6 : Calibration graph of Tolterodine tartarate



iv) Estimation of Formulation

Twenty tablets (Roliten 2mg, Ranbaxy Laboratories Limited., India) were weighed and the average weight was determined. The powder equivalent to 10 mg was transferred to a 100 ml standard flask and extracted with distilled water by shaking for one hour. The solution was filtered through a Whatman filter paper. From this, aliquots of acidic and

alkaline solutions of concentrations 30, 40, 50 µg/ml were prepared. The difference spectras of the appropriately diluted acidic and alkaline solutions were recorded. The amount of Tolterodine tartarate present in the sample solution was computed from the calibration graph. (Table : 4).

Table 4 : Analysis of Formulation

Formulation	Amount (mg/tablet)		% Label claim* ± S.D.
	Labeled	Found	
Roliten	2	1.97	98.3 ± 0.69

* Mean of six determinations

METHOD VALIDATION

i) **Specificity**

The developed method was found to be highly specific as there were no interferences with the excipients in the formulation.

ii) **Linearity and range**

The developed method showed good linear relationship in the concentration range 10-90 µg/ml and the slope, intercept and correlation coefficient was found to be 0.0140, -0.0004 and 0.9999 respectively. Molar absorptivity and Sandell's sensitivity was found to be 5392.01 l/mole/cm and 0.0885 µg/cm/0.001AU respectively.

iii) **Accuracy**

The accuracy of the method was determined through recovery studies. To 10 mg equivalent of Tolterodine tartarate, the standard drug of Tolterodine tartarate was added at 100% level. The recovery procedure was repeated 6 times and the %recovery and %RSD was calculated (Table : 5) using the formula:

$$\% \text{ recovery} = b\text{-}a/\ c \times 100$$

Where, a = amount of drug found before addition of standard drug.

b = amount of drug found after addition of standard drug.

c = amount of standard drug added.

Table : 5 Recovery Studies

Drug	% Recovery*	%RSD
Tolterodine tartarate	102.13	0.94

* Mean of six determinations

Results and Discussion

A simple, precise and rapid difference spectroscopic method was developed for the estimation of Tolterodine tartarate from its tablet dosage form. This method was based on the technique of altering the spectral properties of the analyte by adjusting the pH by means of 0.5M HCl and 0.5M NaOH. There were no interferences found with the excipients in the formulation. The content of Tolterodine tartarate was found to be 1.97 mg/tab. The %recovery was found to be 102.13% ± 0.94 at 100% level which indicated the accuracy and reliability of the method. Hence, the developed method can be used for the routine analysis of Tolterodine tartarate in its tablet dosage form.

INTRODUCTION TO VISIBLE SPECTROSCOPY [3,11-17]

Visible Spectrophotometry is one of the most frequently used techniques in pharmaceutical analysis. It involves the measurement of visible (380-800 nm) radiation by a substance in solution. The variation of colour of a system with change in the concentration of the same components forms the basis of colorimetric analysis. The colour is usually due to the formation of a coloured complex by addition of the appropriate reagent. The intensity of the colour may then be compared with that obtained by treating a known amount of the substances in the same manner. Colorimetry is concerned with the determination of the concentration of a substance by the measurement of relative absorbance of light with respect to a known concentration of the substances.

Chromophore

A chromophore is a group responsible for light absorption by a molecule. Originally, the term chromophore was applied to the system responsible for imparting colour to the compound. Most chromophore therefore contains only one or more multiple bonds. For example, in azo dyes, the aryl conjugated azo group (Ar-N=N-Ar) is clearly the principle chromophore and in nitro compounds, the yellow colour is carried out by NO_2 group.

The term has been retained within an extended interpretation to simply any functional group that absorbs electromagnetic radiation, whether or not a 'colour' is thereby produced. Thus, the carbonyl group is a chromophore.

Auxochrome

An auxochrome was an earlier defined term for a group that could enhance the colour imparting properties of a chromophore without being itself a chromophore. Example: OR, $-NH_2$, $- NR_2$ etc.

When a substance does not possess suitable chromogenic properties, it may be made to react with an absorbing species reagent and converted to an absorbing species. Reagents like Gibbs reagent, Ehrlich's reagent, Folin-ciocalteu's reagent, etc., can be used to convert the substance to a chromogen.

Absorption spectrophotometry in the ultra violet and visible region is considered to be one of the physical methods used for the quantitative analysis and structural elucidation. In developing these new colorimetric methods, the systematic investigations were performed in the following manner.

I. Spectral characteristics of the coloured species

Absorption spectra against a solvent blank in the visible and near ultraviolet range (300-700nm) are recorded for the reagent alone and for the reagent in the presence of various proportions of the drug to be

determined. The proportions are chosen in such a way so that the absorbing species between the limits of the drug in large excess and the reagent are identified, for maximum sensitivity and greatest precision. The wavelength region at which the two solutions shows the largest difference in the absorbance is chosen for use in the determination, and the subtractive absorption spectrum of the coloured species against a reagent blank is obtained experimentally.

II. Effect of pH

The pH is more closely controlled and varied using fixed concentration of the reagent and the drug to be determined (in the presence of known excess of reagent). The absorbance of each solution is measured against the reagent blank at the same pH; after following definite time interval for complete colour development, over a relatively narrow band of wavelength (5-10 nm) near to the wavelength of maximum absorbance of coloured species as determined from the original absorption spectra.

III. Effect of Reagent Concentration

The basis of most spectrophotometric methods is usually

1. A complex formation reaction
2. An oxidation-reduction process or
3. A catalytic effect

In each of the reaction, the absorbance of the coloured species is measured and thus the sensitivity of the method is effected by the reagent (or catalyst) concentration, and therefore it is important to establish the concentration of the reagent, revised at several concentrations of the species to be determined.

IV. Order of Addition, Rate of Colour Formation and Stability

The order in which the reagents are mixed often has a marked effect on the colour reaction and the rate of colour development. The absorbance produced at the optimum wavelength, pH and reagent concentration of the species to be determined are compared for different orders of mixing and colour development times. The plot of absorbance versus development times with the optimum order of addition reveals that the absorbance reaches a constant reproducible maximum value after a relatively short development time, and then remains constant for a considerably longer period. The stability of the colour produced is studied further over periods of time greater than those investigated during the colour development experiments.

V. Effect of Temperature

Many of the reactions employed, on the basis of spectrophotometric determination are temperature dependent. The manner in which the absorbance varies with the temperature at which the colour is developed is

investigated over the range of temperature likely to be encountered in the laboratory.

VI. Nature of Coloured Species

This method most frequently employed to investigate the reagent and drug ratio in a coloured species which forms the basis of a new method is determined through absorbance measurements in solution via the mole ratio, slope ratio and continuous variation procedures. If these methods are not suitable the nature of coloured species is predicted through the information as given in literature.

Efforts are made to extract the coloured species from the aqueous to organic solvents media using common polar and non-polar water-immiscible solvents with a view to get coloured species of better absorbance and stability reducing the interference of other associating constituents. Solvents extraction is proposed only when there are any positive additives.

VII. Calibration curve and optimum concentration range

A calibration curve for the determination of the drug is constructed by measurement of the absorbance developed by known concentrations of the constituents under optimum conditions against reagent blank. A straight line is obtained if Beer's law is followed.

DEVELOPMENT OF VALIDATED VISIBLE SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TOLTERODINE TARTARATE FROM TABLET DOSAGE FORM

1. Principle

Tolterodine tartarate contains a phenolic hydroxyl group which contributed in giving a purple colored chromogen in the presence of MBTH and cerric ammonium sulphate. This reaction is based on the oxidative coupling of Tolterodine tartarate in the presence of Ce(IV). On oxidation with Ce(IV), 3–methyl–2–benzothiazolinone hydrazone hydrochloride (MBTH) loses two electrons and one proton to give an electrophilic intermediate, which has been considered to be the active coupling species. When this intermediate reacts with one of the nucleophilic sites of Tolterodine tartarate by an electrophilic attack, it yields a purple colored complex. The chromogen formed has an absorption maximum of 552 nm. Beer's law is obeyed in the range of 20-120 µg/ml. (Fig. 7, 8)

Fig. 7 : UV Spectrum of Tolterodine tartarate

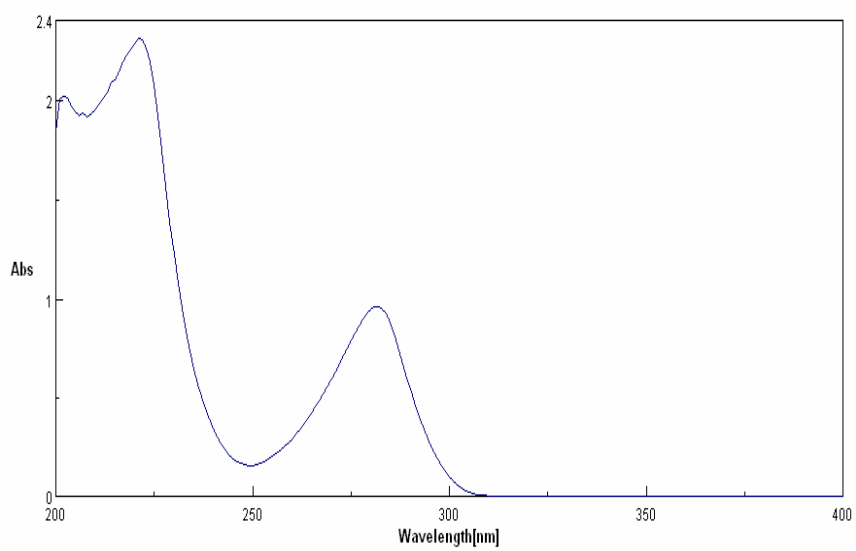
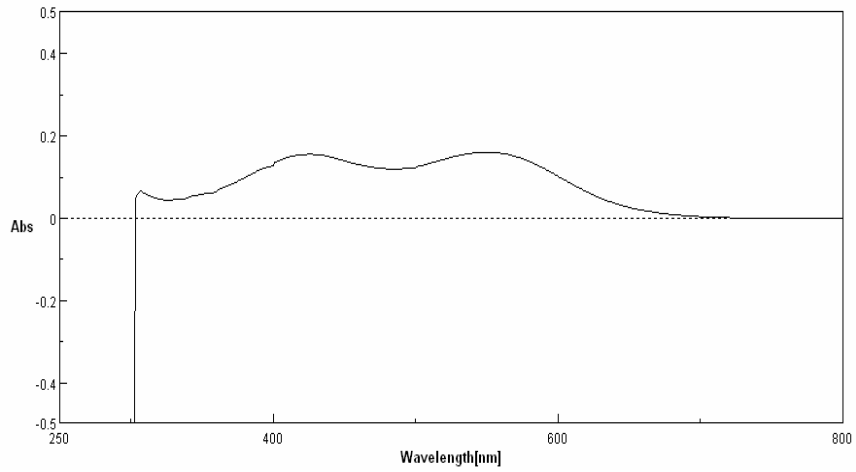


Fig. 8 : Developed chromogen



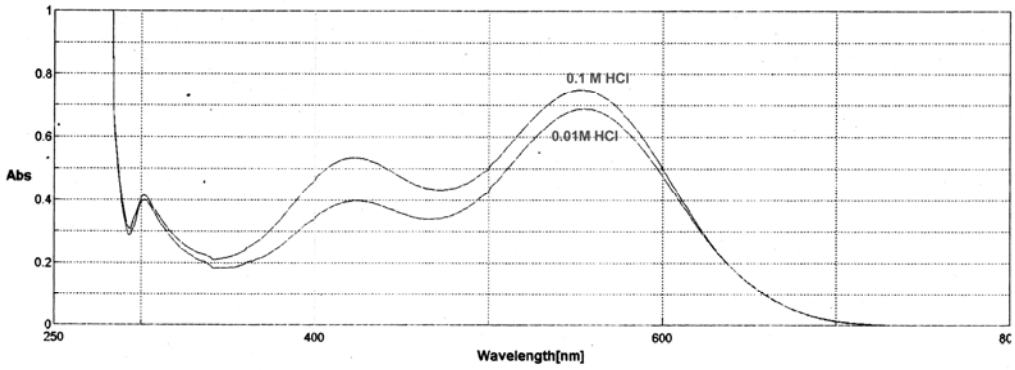
2. Development of the Colorimetric method

a) *Optimization of colorimetric parameters*

➢ **Fixing the molarity of hydrochloric acid for the stock solution**

10 mg of Tolterodine tartarate was added into a series of 10 ml standard flasks and was made upto volume with different molarities of hydrochloric acid such as 0.01M, 0.1M, 0.5M, 1M HCl to get concentrations of 1000µg/ml. The drug did not dissolve in 0.5M and 1M HCl. The drug showed highest absorbance and good spectral pattern in 0.1M HCl. So, 0.1M HCl was selected for the preparation of the standard stock solution (Fig. 9).

Fig.9 : Fixing the molarity of hydrochloric acid



➢ **Fixing the molarity of sulphuric acid for the preparation of cerric ammonium sulphate**

0.003M Cerric ammonium sulphate was prepared by weighing 0.0474 gm of cerric ammonium sulphate into a series of 25 ml standard flasks, dissolved and made upto volume with different molarities of

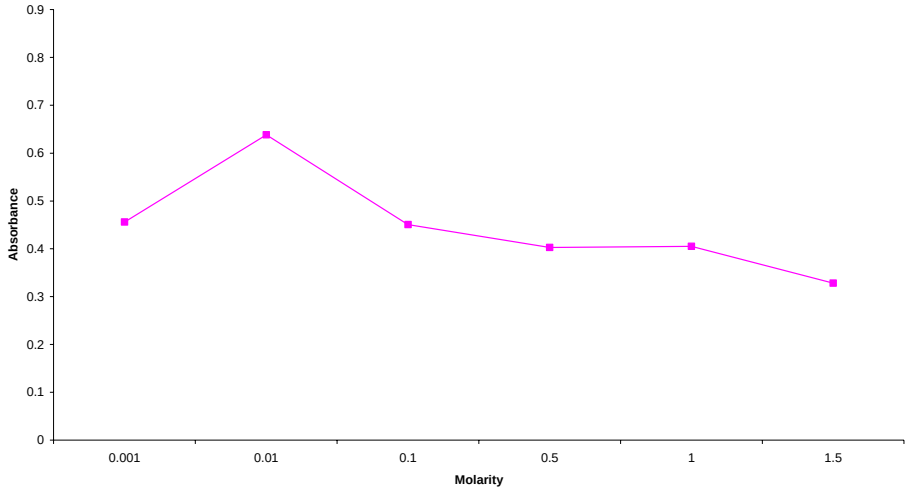
Sulphuric acid such as 0.001M, 0.01M, 0.1M, 0.5M, 1M, 1.5M. Standard Stock Solution of 0.6 ml was added into a series of 10 ml standard flasks. To these, 2 ml of 0.2% MBTH solution and 4 ml of cerric ammonium sulphate dissolved in different molarities of Sulphuric acid were added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The absorbances of these solutions were read against reagent blank (Table : 6). The solution showed highest absorbance and good spectral pattern with 0.003M cerric ammonium sulphate dissolved in 0.01M Sulphuric acid (Fig. 10).

Table 6 : Fixing the molarity of Sulphuric acid

Molarity of Sulphuric acid	Absorbance
0.001M	0.4560
0.01M*	**0.6382**
0.1M	0.4505
0.5M	0.4027
1M	0.4051
1.5M	0.3279

* Selected parameter

Fig.10 : Fixing the Molarity of Sulphuric acid



➢ **Fixing the strength of cerric ammonium sulphate**

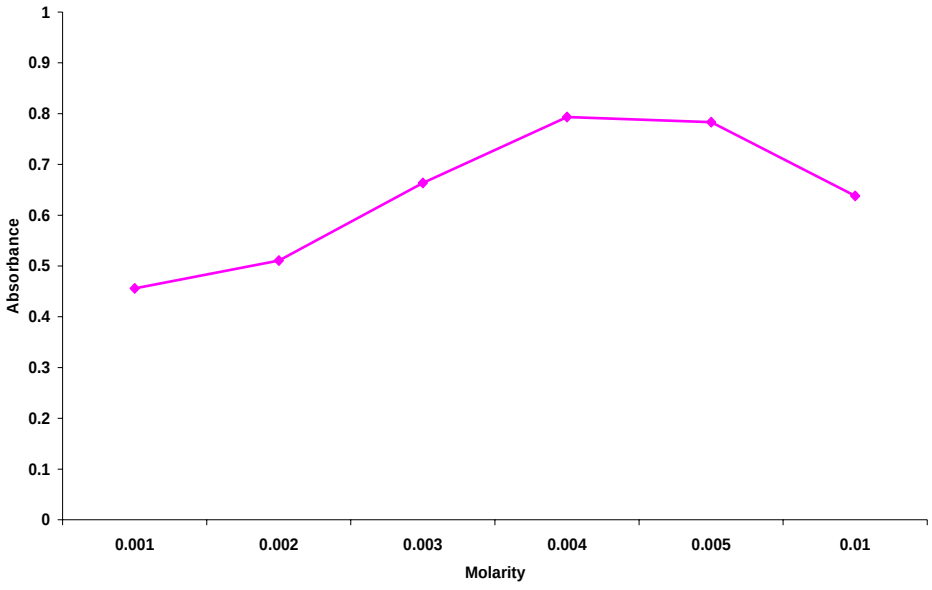
Standard Stock Solution of 0.6 ml was added into a series of 10 ml standard flasks. To these, 2 ml of 0.2% MBTH solution and 4 ml of different molarities of cerric ammonium sulphate such as 0.001M, 0.002M, 0.003M, 0.004M, 0.005M and 0.01M were added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The absorbance's of these solutions were read against reagent blank (Table : 7). The solution showed highest absorbance and good spectral pattern with 0.004M Cerric Ammonium Sulphate (Fig.11).

Table : 7 Fixing the strength of cerric ammonium sulphate

Strength of cerric ammonium sulphate	Absorbance
0.001M	0.4561
0.002M	0.5109
0.003M	0.6635
0.004M*	**0.7935**
0.005M	0.7832
0.01M	0.6382

* Selected parameter

Fig.11 : Fixing the strength of cerric ammonium sulphate



➢ **Fixing the volume of cerric ammonium sulphate**

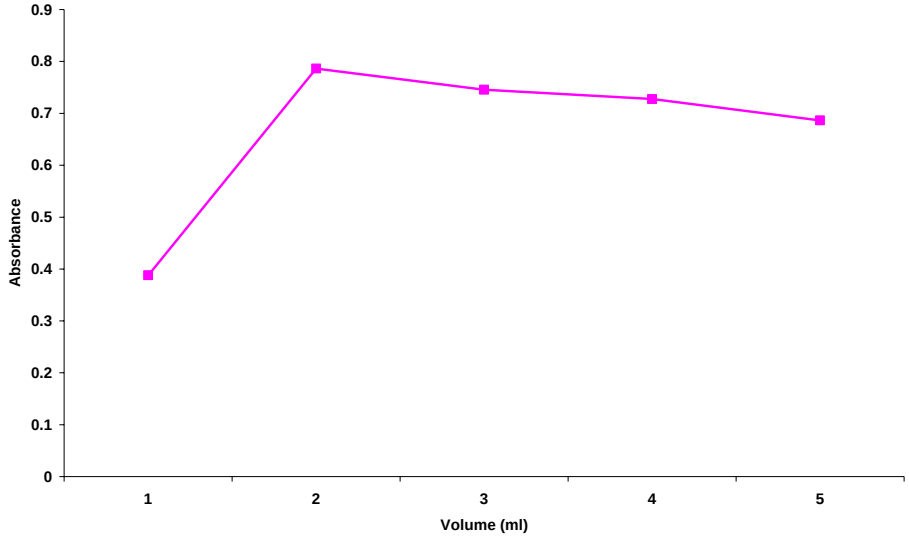
Standard Stock Solution of 0.6 ml was added into a series of 10 ml standard flasks. To these, 2 ml of 0.2% MBTH solution and different volumes of 0.004M cerric ammonium sulphate such as 1, 2, 3, 4, 5 ml were added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The absorbances of these solutions were read against reagent blank (Table : 8). The solution showed highest absorbance and good spectral pattern with 2 ml of 0.004M cerric ammonium sulphate (Fig. 12).

Table 8 : Fixing the volume of cerric ammonium sulphate

Volume of cerric ammonium sulphate (ml)	Absorbance
1	0.3881
2*	**0.7862**
3	0.7454
4	0.7275
5	0.6864

***** Selected parameter

Fig. 12 Fixing the volume of cerric ammonium sulphate



➢ **Fixing the strength of MBTH solution**

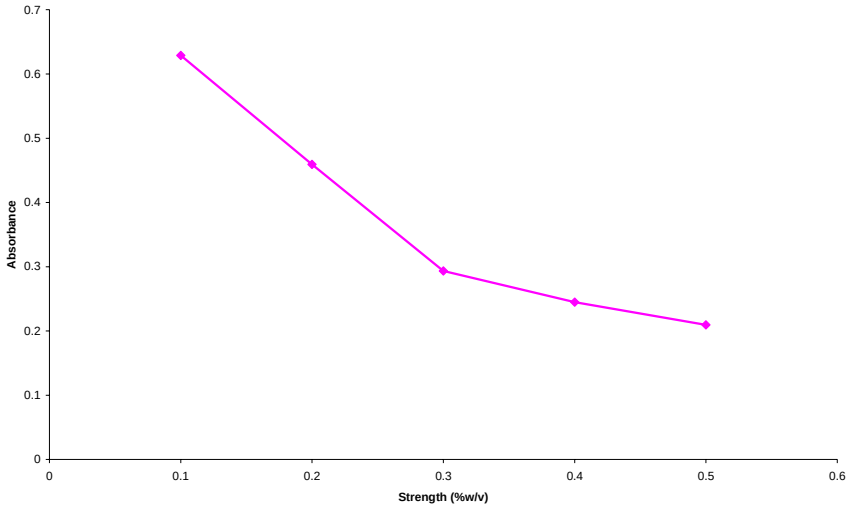
Standard Stock Solution of 0.6 ml was added into a series of 10 ml standard flasks. To these, 2 ml of different concentrations of MBTH solutions such as 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and 2 ml of 0.004M cerric ammonium sulphate were added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The absorbances of these solutions were read against reagent blank. The solution showed highest absorbance and good spectral pattern with 0.1% MBTH solution (Table 9 & Fig.13).

Table 9 : Fixing the strength of MBTH

Strength of MBTH (%w/v)	Absorbance
0.1*	**0.6288**
0.2	0.4594
0.3	0.2933
0.4	0.2448
0.5	0.2096

* Selected parameter

Fig. 13 : Fixing the strength of MBTH



➢ **Fixing the volume of MBTH solution**

Standard Stock Solution of 0.6 ml was added into a series of 10 ml standard flasks. To these, different volumes of 0.1% MBTH solution such as 0.05, 1, 2, 3, 4, 5 ml and 2 ml of 0.004M cerric ammonium sulphate were added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The

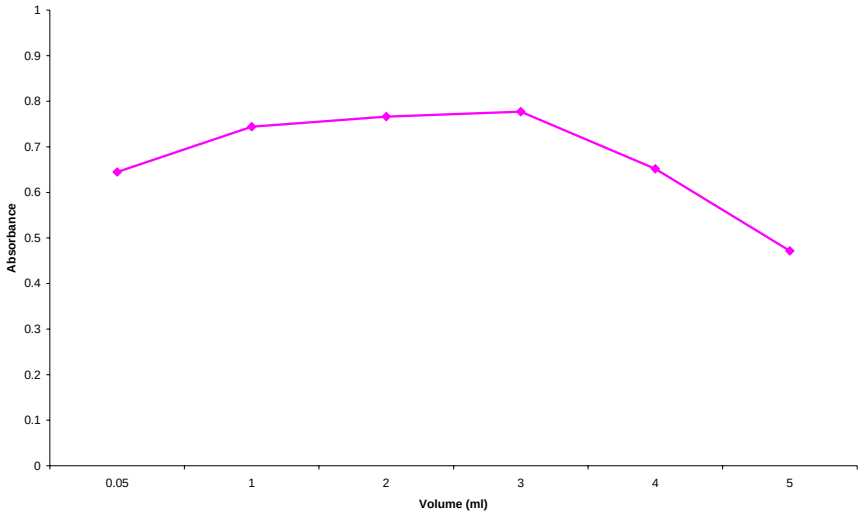
absorbances of these solutions were read against reagent blank (Table 10). The solution showed highest absorbance and good spectral pattern with 3 ml of 0.1% MBTH solution (Fig. 14).

Table 10 : Fixing the volume of MBTH

Volume of MBTH (ml)	Absorbance
0.05	0.6449
1	0.7441
2	0.7662
3*	**0.7771**
4	0.6516
5	0.4718

* Selected parameter

Fig. 14 : Fixing the volume of MBTH



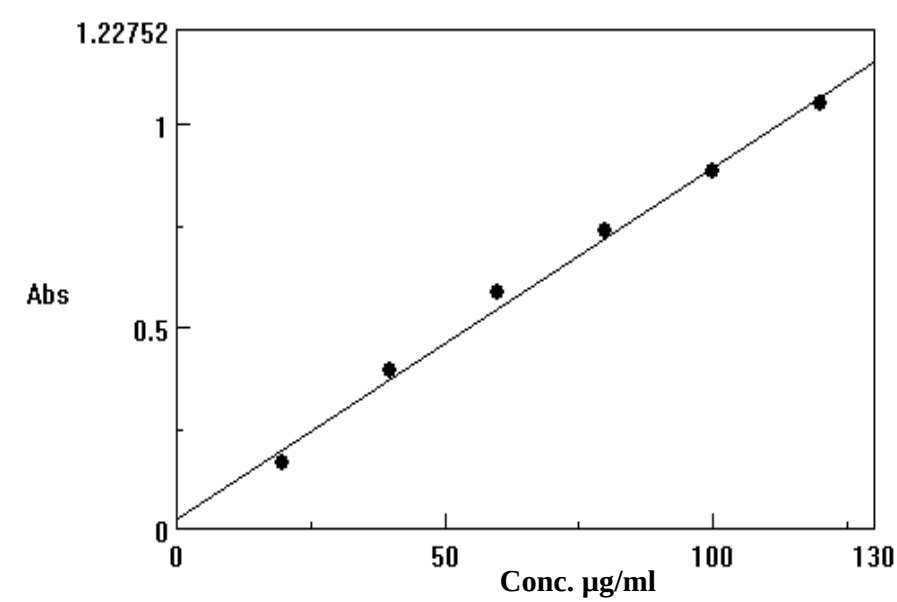
b) ***Preparation of standard graph***

Aliquots of standard stock solution of volume 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 ml were added into a series of 10 ml standard flasks. To these, 3 ml of 0.1% MBTH solution and 2 ml of 0.004M cerric ammonium sulphate was added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The absorbances of these solutions were noted at 552 nm against reagent blank. A standard graph was obtained by plotting concentration of standard solutions Vs absorbance (Fig. 15). The slope, intercept and correlation coefficient were found to be 0.0087, 0.0279 and 0.9970 respectively. Tolterodine tartarate showed good linear relationship in the concentration range 20-120 µg/ml with the molar absorptivity of 4314.37 l/mole/cm and the Sandell's sensitivity was found to be 0.1106 µg /cm/0.001AU (Table : 11).

Table 11 : Absorbance values of Tolterodine tartarate

Concentration (µg/ml)	Absorbance at 552 nm
20	0.1654
40	0.3893
60	0.5813
80	0.7325
100	0.8831
120	1.0505

Fig. 15 : Calibration graph of Tolterodine tartarate



Analysis of Formulation

20 tablets (Roliten, Ranbaxy Laboratories Limited, India) were weighed and the average weight was calculated. A quantity equivalent to 10 mg of Tolterodine tartarate was weighed and transferred to a 10 ml standard flask, dissolved in 0.1M HCl and made upto volume with the same. It was then filtered through Whatman filter paper. From this stock solution, 0.6 and 0.8 ml were pipetted into 10 ml standard flasks. To these, 3 ml of 0.1% MBTH solution and 2 ml of 0.004M cerric ammonium sulphate was added. The solutions were kept aside for 30 minutes to favor complex formation and made upto volume with distilled water. The absorbances of these solutions were noted at 552 nm against reagent

blank and the amount of drug present in the formulation was calculated (Table :12) by single-point standardization method and the equation is as follows:

$$c_{test} = \frac{A_{test} \times c_{std}}{A_{std}}$$

Table 12 : Analysis of Formulation

Formulation	Amount(mg/tablet)		%Label Claim*± S.D
	Labeled	Found	
Roliten	2	1.97	98.5±0.32

*Mean of six determinations

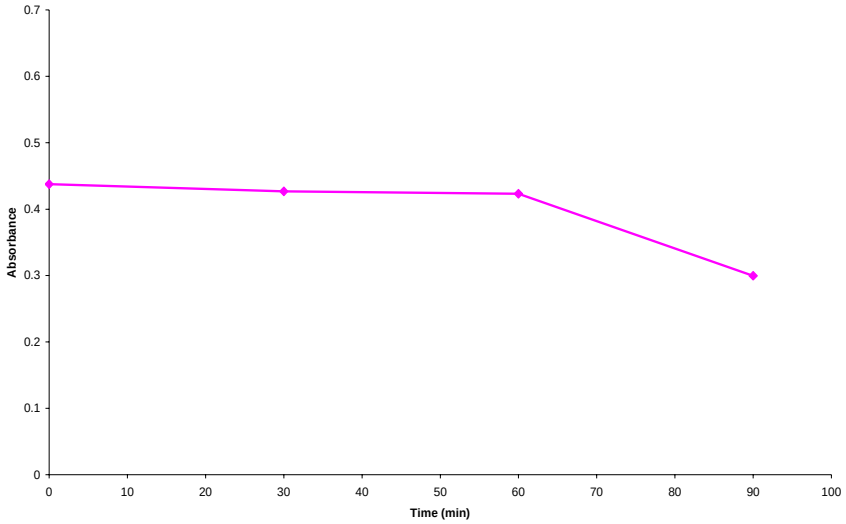
a) Stability studies

Stability studies were carried out at room temperature and the absorbance of the chromogen was measured with respect to time (Table 13) The color was found to be stable for 1 hour (Fig. 16).

Table 13 : Stability Studies

Time(min)	Absorbance
0	0.4376
30	0.4268
60	0.4232
90	0.2997

Fig. 16 : Stability Studies



METHOD VALIDATION

The developed method was validated as per ICH Guidelines in terms of specificity, linearity, range, precision and accuracy.

i) Accuracy

Accuracy of the developed method was determined by conducting the recovery studies. To the powder formulation equivalent to 10 mg of Tolterodine tartarate, standard drug of Tolterodine tartarate was added at 100% level. The concentration of the drug present in the resulting solutions was determined by the proposed method. The recovery procedure was repeated six times and the %recovery was calculated (Table : 14).

Table 14 : Recovery Studies

Formulation	%Recovery*	% RSD
Roliten	98.3	0.76

*Mean of six determinations

ii) Specificity

The developed method was found to be highly specific as there were no interferences found with the excipients used in the formulation.

iii) Precision

Precision of the method was determined by the repeatability studies. Intra-day and inter-day assays were carried out with the method by repeating the procedure six times and the %RSD was calculated. The datas are shown in (Table : 15 and 16).

Table 15 : Intra-day Precision

Drug	Concentration (µg/ml)	Absorbance	%RSD*
Tolterodine tartarate	40	0.3893	0.12
		0.3890	
		0.3892	
		0.3896	
		0.3899	
		0.3892	

*Mean of six determinations

Table 16 : Inter-day Precision

Drug	Concentration (µg/ml)	Days	Absorbance	%RSD*
Tolterodine tartarate	40	1st	0.3893	0.12
			0.3890	
			0.3892	
			0.3896	
			0.3899	
			0.3892	
		2nd	0.3895	0.24
			0.3893	
			0.3891	
			0.3897	
			0.3892	
			0.3893	

*Mean of six determinations

iv) Linearity and Range

Tolterodine tartarate showed good linearity in the concentration range of 20 – 120 µg/ml and the slope, intercept and correlation coefficient was found to be 0.0087, 0.0279 and 0.9970 respectively. Molar absorptivity was found to be 4314.37 l/mole/cm and Sandell's sensitivity was found to be 0.1106 µg/cm/0.001AU.

Results and Discussion

The results obtained by the proposed colorimetric method for the estimation of Tolterodine tartarate in the tablet dosage form showed that the amount recovered was in good agreement with the label claim of the formulation. The method was simple, precise and accurate and can be employed for the routine analysis of Tolterodine tartarate in the tablet dosage form. The recovery of the sample was close to 100% indicating the accuracy, reliability and reproducibility of the method.

INTRODUCTION TO HPTLC[18-22]

HPTLC is the most simple separation technique today available to the analyst. HPTLC is a qualitative tool for separation of simple mixtures where speed, low cost and simplicity are required and it is also a tool for quantitative analysis with high sample throughput.

Selection of chromatographic layer

Pre-coated plates with different support material, sorbent layer and with different sorbent thickness of 100-250μm are used for quantitative and qualitative analysis.

Pre-washing and activation of pre-coated plates

Sorbents on the plate absorb water vapour, impurities and other volatile substances from atmosphere which gives dirty zones on development and fail to give reproducible results. The plates are cleaned by a method referred to as pre- washing.

The plates are activated by drying in drying cupboards for sufficient time to ensure removal of the washing liquids (Usually, for methanol 30-60 min at 105°). Plates are then stored in dust free atmosphere under ambient condition.

Sample preparation

This procedure involves, dissolving the dosage form in a solvent with complete recovery of intact compounds of interest and minimum of matrix with suitable concentration of analyte during extraction and analysis must be considered and ensured.

Sample application

Sample application is one of the most critical step for obtaining good resolution for quantification by HPTLC. The sample should be applied through clean upper end of the capillary to marked point. Usually application of 1-10 μl for HPTLC is recommended. Samples are applied as bands as it offers better separation, higher response of densitometer, smaller spot broadening etc.

Mobile phase

Mobile phase (Solvent system) is selected by trial and error method. Mobile phase should be chosen by taking into consideration chemical properties of analytes and the sorbent layer. Mobile phase containing more than 3 or 4 components should be avoided as it is often difficult to get reproducible ratios.

Preconditioning (Chamber saturation)

In a saturated chamber, the solvent vapors get uniformly distributed throughout the chamber. As soon as the plate is placed in a

saturated chamber it soon gets pre loaded with solvent vapour, hence less solvent is only required, resulting in lower R_f values.

Development and drying

Ascending, descending, 2-dimensional, horizontal, gradient, radial, multidimensional etc. are the most common mode of chromatographic development. Rectangular glass chambers, twin-trough chambers, V-shaped chambers etc. are commonly used for carrying out different types of TLC development.

Detection and visualization

The characteristic features of TLC/ HPTLC is the possibility to utilize post chromatographic off line derivatisation. Detection under UV light is the most preferred method as it is non-destructive and commonly employed for densitometric scanning.

Criteria for selection of most suitable wave length

The light intensity remitted by chromatographic zones is usually lower than the sorbent layer around it. Therefore, absorption spectra of a compound can directly be determined on HPTLC plate itself in comparison to substance free portion of sorbent layer.

Scanning is mostly carried out at a wave length of maximum absorption because the difference between, absorption by the

chromatographic zone and the blank area of sorbent layer around it is the largest, background being least.

In case of complex formulations, the analyst should explore the possibility of selecting a single wavelength at which the entire chromatogram could be scanned referred to as “most suitable wavelength”.

While selecting single wavelength, the interest of minor components in the formulation needs special consideration.

METHOD DEVELOPMENT AND VALIDATION OF TOLTERODINE TARTARATE IN TABLET DOSAGE FORM BY HPTLC

1. Selection of Solvent

The drug is dissolved in a solvent in which it is soluble and in which the drug showed good stability. Another criteria for the selection of solvent is that it has to be volatile, cheap and easily available. As Tolterodine tartarate is readily soluble in methanol and showed good stability, it was selected as the solvent.

2 Selection of Detection Wavelength

An ideal wavelength is the one that gives maximum absorption and good response for the drug to be detected. UV spectrum of the drug showed maximum absorption at 282 nm which was selected as the detection wavelength. (Fig .17).

3. Optimization of the Mobile phase

Based on the property of polarity, solubility, various mobile phases were tried.

- The spot did not migrate with **toluene: chloroform: glacial acetic acid (8:2:0.5 %v/v/v).**
- The spot did not migrate with **toluene: ethyl acetate: glacial acetic acid (8:2:0.5 %v/v/v).**
- With **methanol: chloroform: ethyl acetate (7:3:0.5 %v/v/v),** broad peak was observed.

- With **methanol: chloroform: glacial acetic acid (9:0.9:0.1%v/v/v),** a good symmetrical peak was observed.
- With **methanol: n-butanol (8:2 %v/v),** the spot migrated with the solvent front.
- With **n-butanol: glacial acetic acid: ammonia (6:4:0.02%v/v/v),** asymmetric peak was observed.

Out of all the mobile phases tried, **methanol: chloroform: glacial acetic acid (9:0.9:0.1 %v/v/v) gave an acceptable peak with an R_f value of 0.73±0.02**. Some of the chromatograms are shown in Fig. 18-20.

4. Optimization of the mobile phase ratio

Standard stock solution was applied on to the pre-coated TLC plates and developed with different ratios of mobile phase such as 7:2.9:0.1, 8:1.9:0.1 and 9:0.9:0.1 %v/v/v and the chromatograms were recorded. **Tolterodine tartarate gave an acceptable peak with 9:0.9:0.1 %v/v/v with an R_f value of 0.73 ±0.02.**(Fig. 21-23).

5. Fixed chromatographic parameters

Stationary phase	:	Pre-coated silica gel $60F_{254}$ on Aluminium sheets
Mobile phase	:	Methanol: Chloroform: Glacial acetic acid (9:0.9:0.1 %v/v/v)
Chamber Saturation	:	20 minutes

Migration Distance : 85 mm

Band width : 6 mm

Slit dimension : 5 x 0.45 mm

Source of Radiation : Deuterium lamp

Scanning Wavelength : 282 nm

R_f value : 0.73 ± 0.02

6. Preparation of standard stock solution

Standard stock solution was prepared by weighing 10 mg of Tolterodine tartarate into a 10 ml standard flask and the volume was made upto 10 ml with methanol to get a concentration of 1000 µg/ml. From this, 3 ml of solution was pipetted into a 10 ml standard flask and made upto volume with methanol to get a concentration of 300 µg/ml.

7. Preparation of standard graph

From the Stock solution, 1 to 5 µl was spotted on the TLC plate, followed by development and scanning the developed spots .The peak areas were recorded (Table : 17). Calibration graph was obtained by plotting the peak areas against the corresponding concentration of the standard solutions (Fig. 24-29).

Table 17 : Peak area values of Tolterodine tartarate

Concentration (ng/spot)	Peak area
300	369.4
600	959.8
900	1518.5
1200	1878.4
1500	2560.4

8. Analysis of formulation

20 tablets (Roliten, Ranbaxy Laboratories Limited., Mumbai), each containing 2 mg of Tolterodine tartarate were taken, weighed and a quantity equivalent to 10 mg of Tolterodine tartarate was taken in a 10 ml standard flask and made upto volume with methanol, extracted by shaking and then filtered through a Whatman filter paper. From this solution, 3 ml was pipetted out into a 10 ml standard flask and made upto volume with methanol to get the concentration of 300 µg/ml .The formulation was assayed by spotting 3 µl onto the plate followed by development and scanning. The peak areas were recorded (Fig. 30) and the amount present was found (Table : 18) from the linear regression analysis and the equation as follows :

$$y = \alpha + \beta x$$

$$\alpha = \frac{(\sum y)(\sum x^2) - (\sum x)(\sum xy)}{N\sum x^2 - (\sum x)^2}$$

$$\beta = \frac{N\sum xy - (\sum x)(\sum y)}{N\sum X^2 - (\sum x)^2}$$

Where, x = concentration

y = absorbance

N = number of pairs of values

Table 18 : Analysis of Formulation

Drug	Amount(mg/tablet)		% Label claim* ± S.D
	Labeled	Found	
Tolterodine tartarate	2	1.99	99.6 ± 0.58

* Mean of six determinations

METHOD VALIDATION

The developed method was validated as per ICH guidelines in terms of linearity, accuracy, limit of detection, limit of quantification, precision and stability.

1. Linearity and Range

Aliquots of standard solutions of Tolterodine tartarate were applied on the pre-coated silica gel $60F_{254}$ aluminium sheet. The plate

was dried, developed and analyzed .The linear regression data showed a good linear relationship over a concentration range of 300-1500 ng/spot. The slope, intercept and correlation coefficient were found to be 1.626, 48.12 and 0.9993 respectively.

2. Accuracy

The accuracy of the method was determined by conducting the recovery studies. Recovery studies were carried out at 50% and 100% levels. To 10mg equivalent of Tolterodine tartarate from tablet formulation, standard drug of Tolterodine tartarate was added at 50% and 100% levels. The percentage recovery and %RSD were calculated. (Table : 19)

Table 19 : Recovery studies

Drug	%Recovery*		%RSD	
	50%	100%	50%	100%
Tolterodine tartarate	99.02	99.46	0.86	1.04

***** Mean of six determinations

3. Precision

Precision of the method was determined by:

- Intra-day Precision
- Inter-day Precision
- Repeatability

⇨ Repeatability of Sample application

⇨ Repeatability of Measurement

Intra-day Precision

Intra-day Precision was found out by carrying out the analysis of the standard drug at two different concentrations- 900 and 1200 ng/spot for four times on the same day. Each concentration was applied in triplicate and the %RSD was calculated. (Table : 20)

Table 20 : Intra-day Precision

Drug	Concentration (ng/spot)	Peak area	%RSD*
Tolterodine tartarate	900	1524.6	0.52
		1512.8	
		1513.3	
		1505.5	
	1200	1860.2	0.91
		1862.7	
		1826.9	
		1841.2	

* Mean of four determinations

Inter-day precision

Inter-day Precision was determined by carrying out the analysis of the standard drug at two different concentrations – 900 and 1200 ng/spot for two days and the %RSD was calculated. (Table : 21)

Table 21 : Inter-day Precision

Drug	Concentration (ng/spot)	Days	Peak area	%RSD*
Tolterodine tartarate	900	1st	1524.6	0.52
			1512.8	
			1513.3	
			1505.5	
		2nd	1534.0	0.33
			1522.0	
			1530.3	
			1530.3	
	1200	1st	1860.2	0.91
			1862.7	
			1826.9	
			1841.2	
		2nd	1862.7	0.23
			1864.2	
			1864.2	
			1872.9	

***Mean of four determinations

Repeatability

Repeatability of Sample application

Repeatability of sample application was determined by spotting 900 and 1200 ng/spot of drug solution on a pre-coated TLC plate six times followed by development, scanning and the %RSD was calculated. (Table : 22)

Table 22 : Repeatability of Sample application

Drug	Concentration (ng/spot)	Peak area	%RSD*
Tolterodine tartarate	900	1553.7	0.91
		1546.2	
		1530.5	
		1563.0	
		1558.6	
		1551.7	
	1200	1865.4	1.54
		1861.2	
		1871.1	
		1802.0	
		1822.2	
		1863.7	

***Mean of six determinations

Repeatability of Measurement

Repeatability of measurement was determined by spotting 900 and 1200ng/spot of drug solution on a pre-coated TLC plate and developed. The plate was then scanned six times without changing the position of the plate and the %RSD was calculated. (Table : 23)

Table 23 : Repeatability of Measurement

Drug	Concentration ng/spot	Peak area	%RSD*
Tolterodine tartarate	900	1530.5	1.57
		1535.7	
		1544.0	
		1539.2	
		1531.9	
		1536.2	
	1200	1877.9	0.19
		1875.1	
		1869.2	
		1879.3	
		1873.0	
		1870.4	

* Mean of six determinations

4. Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ were determined by applying decreasing amount of the drug in triplicate on the plate. The lowest concentration at which the peak is detected is called **Limit of Detection** and it was found to be 180 ng/spot. (Fig. 31) The lowest concentration at which the peak is quantified is called **Limit of Quantification** and it was found to be 300 ng/spot. (Fig. 32).

5. Stability Studies

When the developed chromatographic plate is exposed to the atmosphere, the analyte is likely to decompose. Hence, it is necessary to conduct the stability studies on the drug. Stability of Tolterodine tartarate on the plate was studied at different intervals and the peak areas were recorded. The developed plate was stable for three hours which was indicated by the reduction in the peak areas.

(Table : 24)

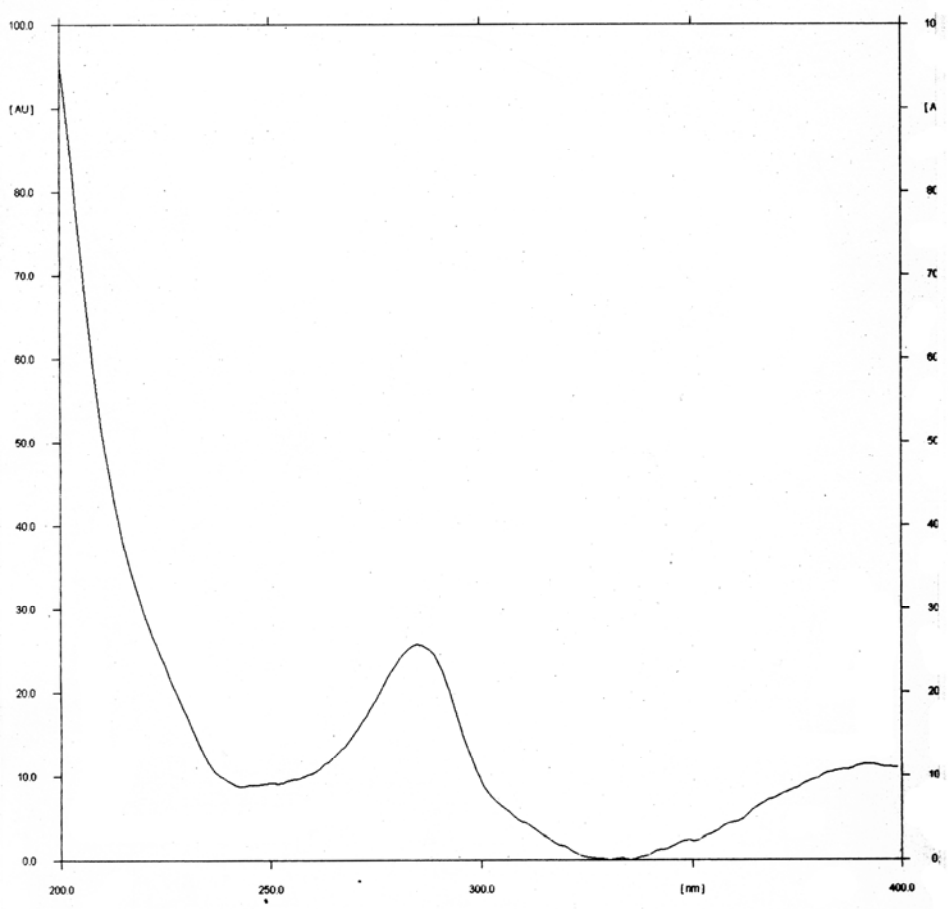
Table 24 : Stability Studies

Drug	Concentration ng/spot	Time (hours)	Peak area
Tolterodine tartarate	900	0	1867.7
		1/2	1836.1
		1	1821.2
		2	1801.0
		3	1761.3

Results and Discussion

The method developed was specific and there was no additional peaks observed for sample drug formulation. From the % RSD, it was observed that the method was precise. The content of Tolterodine Tartarate was found to be 1.99 mg/tab. The %recovery was found to be 99.4± 1.04 at 100% level and 99.02±0.86 at 50%level. This indicated that the method was very accurate. Stability studies proved that the drug was stable for three hours after scanning. So, the method can be used for routine analysis of Tolterodine tartarate in its tablet dosage form.

Fig. 17 : UV spectrum of Tolterodine tartarate on TLC plate



SELECTION OF MOBILE PHASE

Fig. 18: Methanol : Chloroform : Ethyl acetate (7: 3: 0.5 %v/v/v)

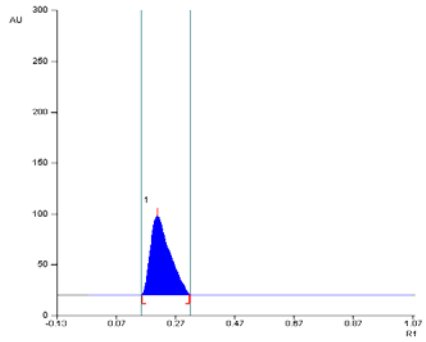


Fig. 19: n-butanol : Glacial acetic acid : Ammonia

(6: 4: 0.02 %v/v/v)

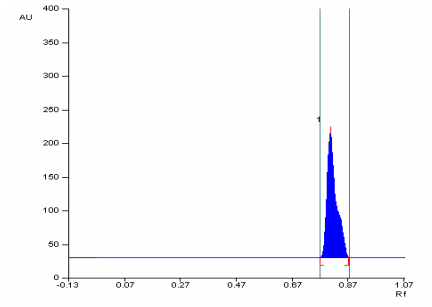
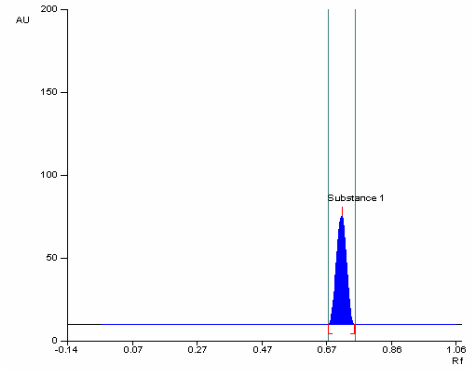


Fig. 20:Methanol: Chloroform: Glacial acetic acid (9: 0.9: 0.1 %v/v/v)



EFFECT OF RATIO OF MOBILE PHASE

Fig. 21: Methanol: Chloroform: Glacial acetic acid (7: 2.9: 0.1 %v/v/v)

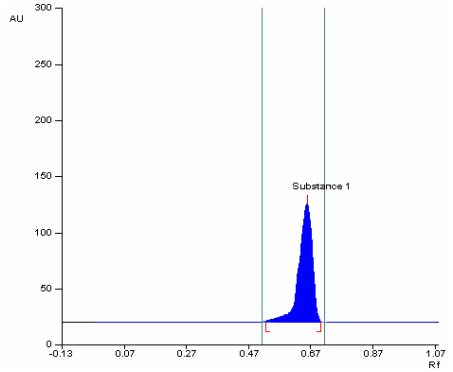


Fig. 22: Methanol: Chloroform: Glacial acetic acid (8: 1.9: 0.1 %v/v/v)

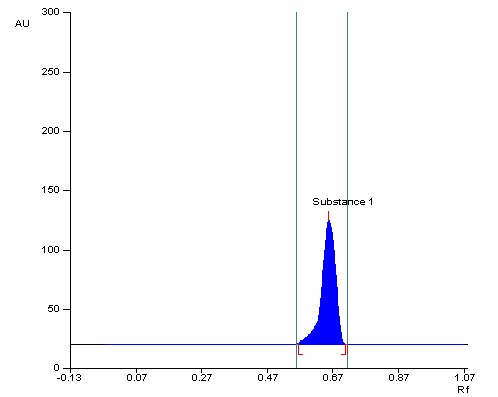
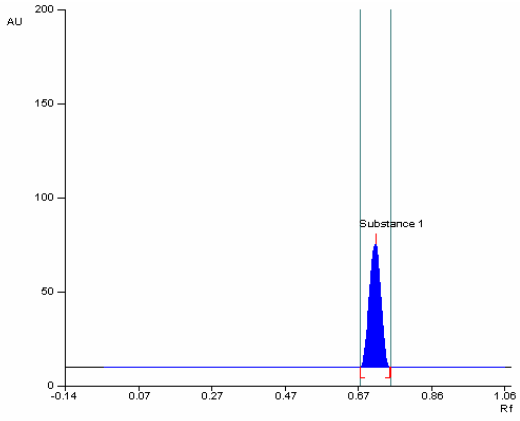


Fig. 23: Methanol: Chloroform: Glacial acetic acid (9: 0.9: 0.1 %v/v/v)



CHROMATOGRAM OF STANDARDS

Fig. 24: 300 ng /spot

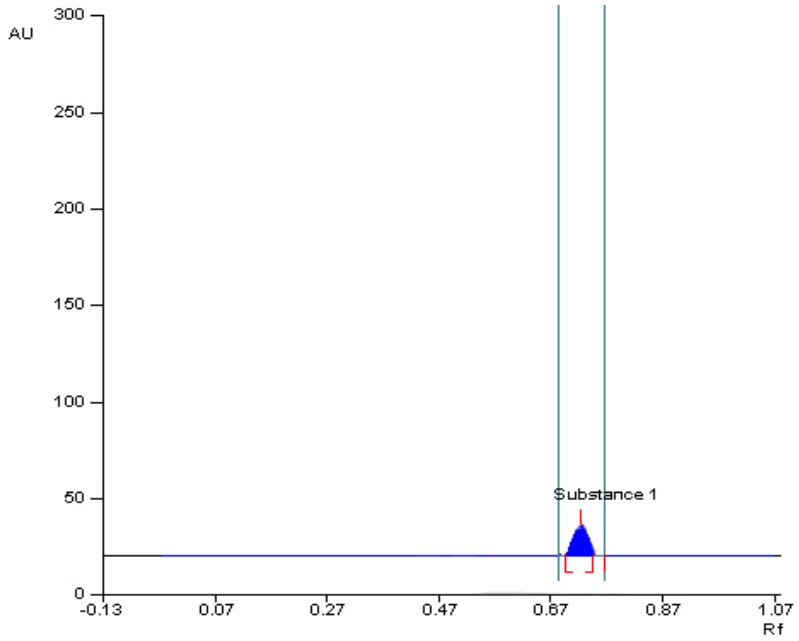


Fig. 25: 600 ng /spot

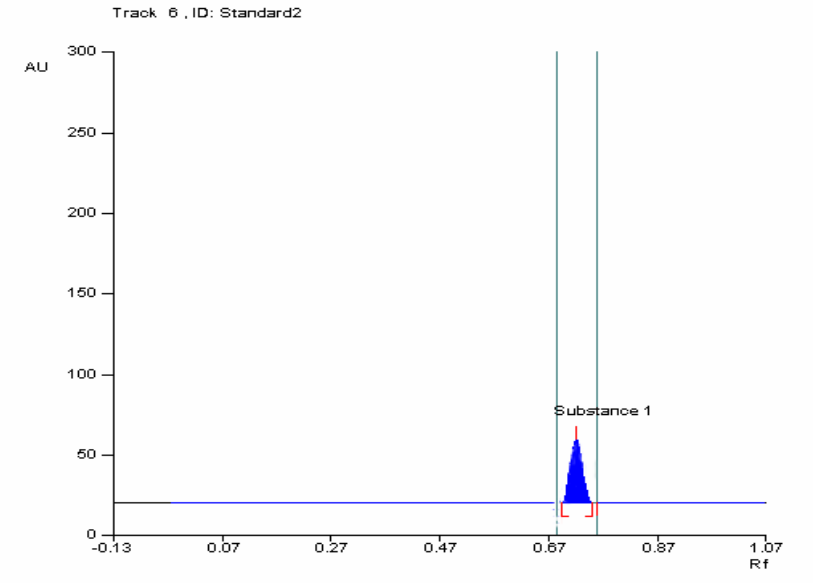


Fig. 26: 900 ng /spot

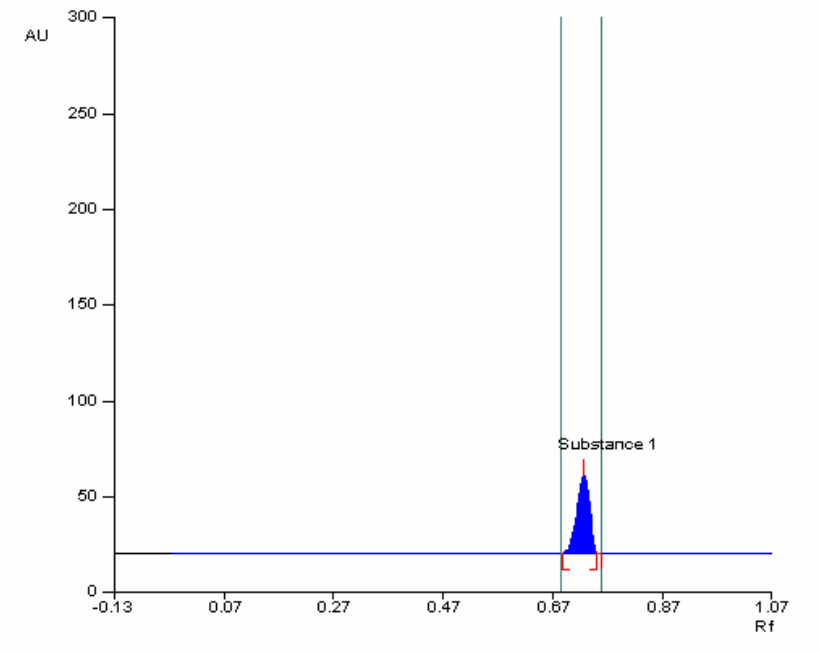


Fig. 27: 1200 ng /spot

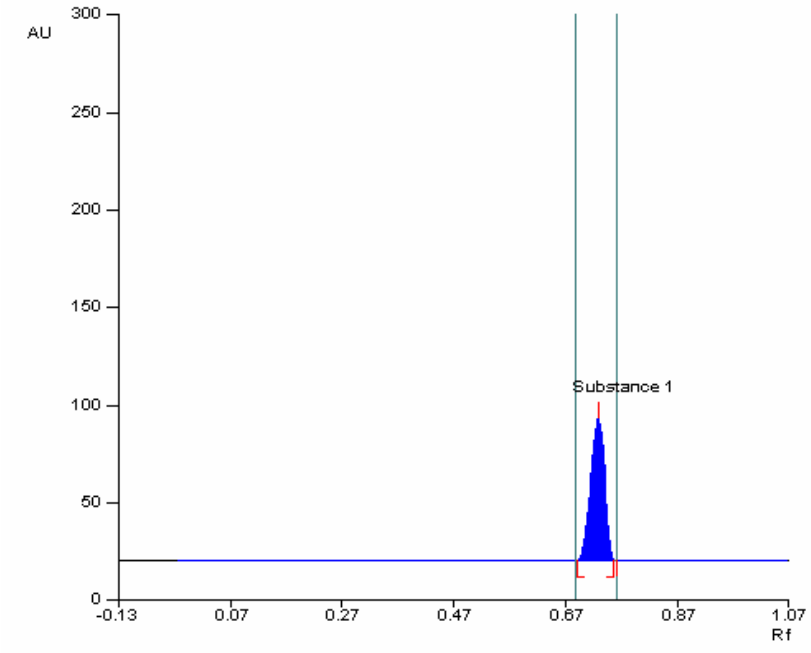


Fig. 28: 1500 ng /spot

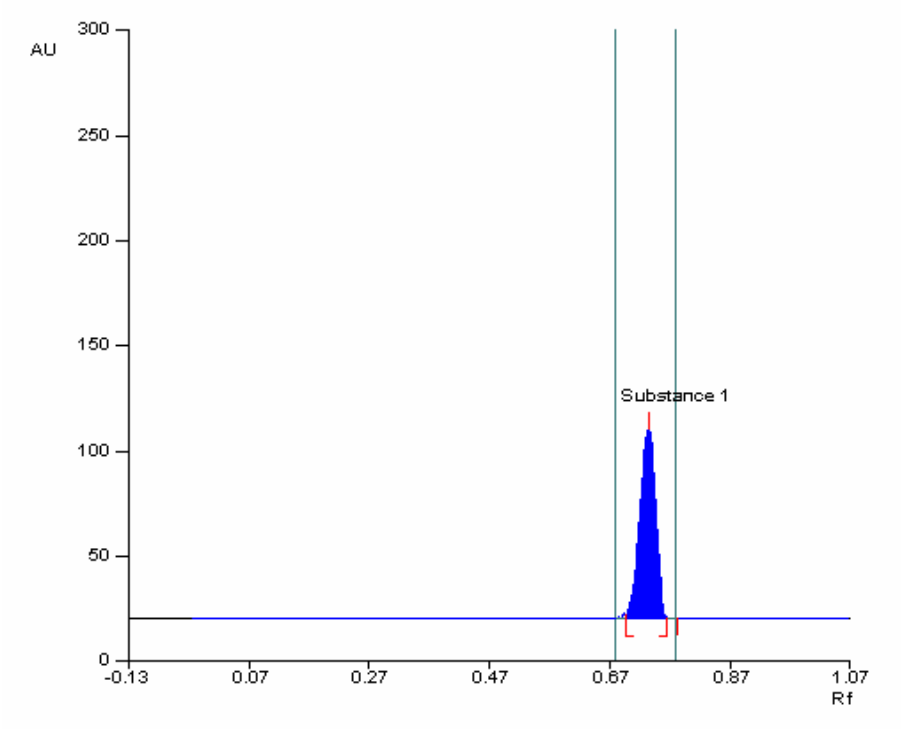


Fig. 29: Calibration graph of Tolterodine tartarate

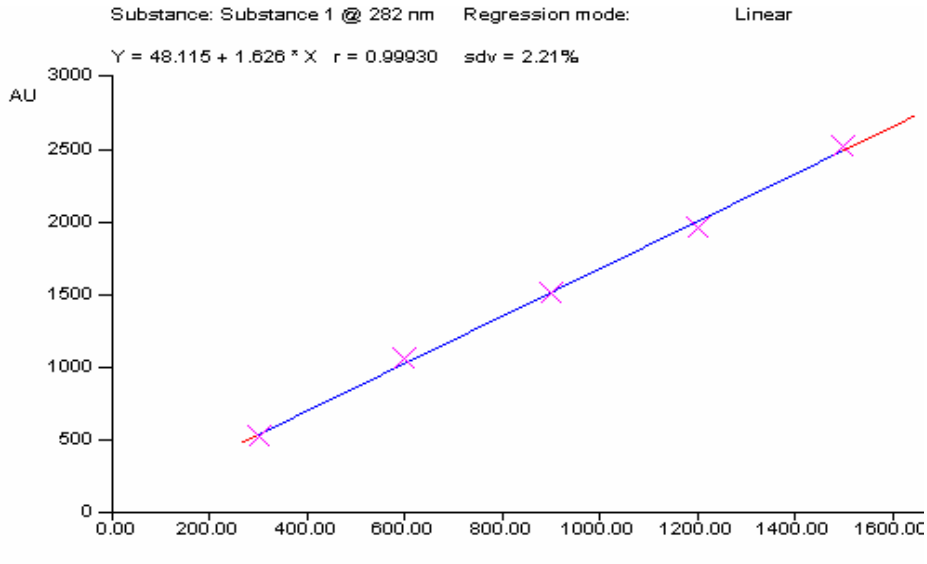


Fig. 30: Chromatogram of formulation

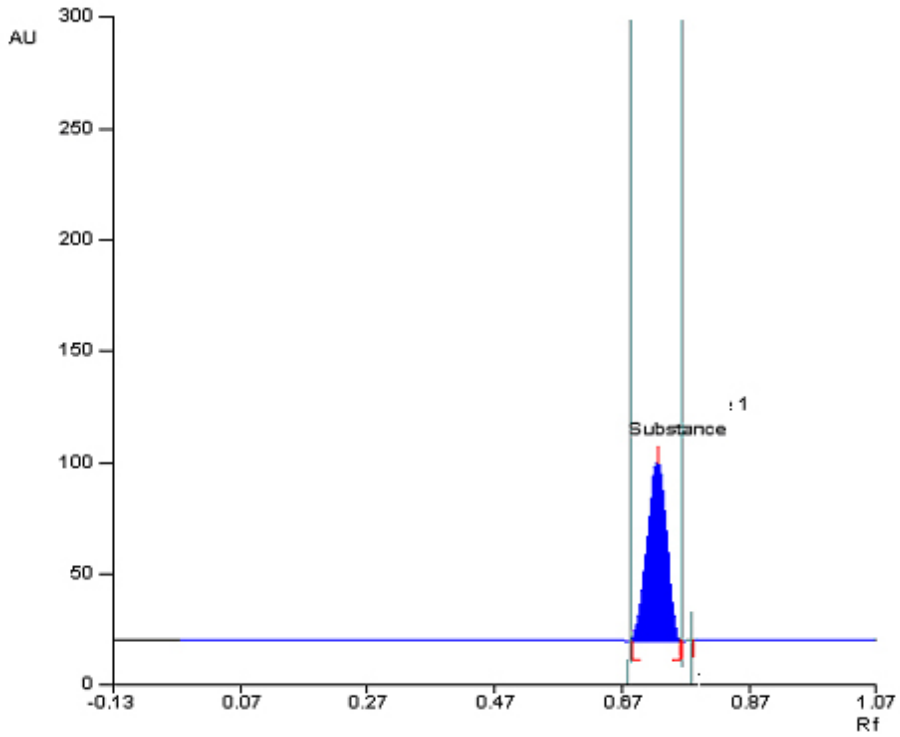


Fig. 31: LOD

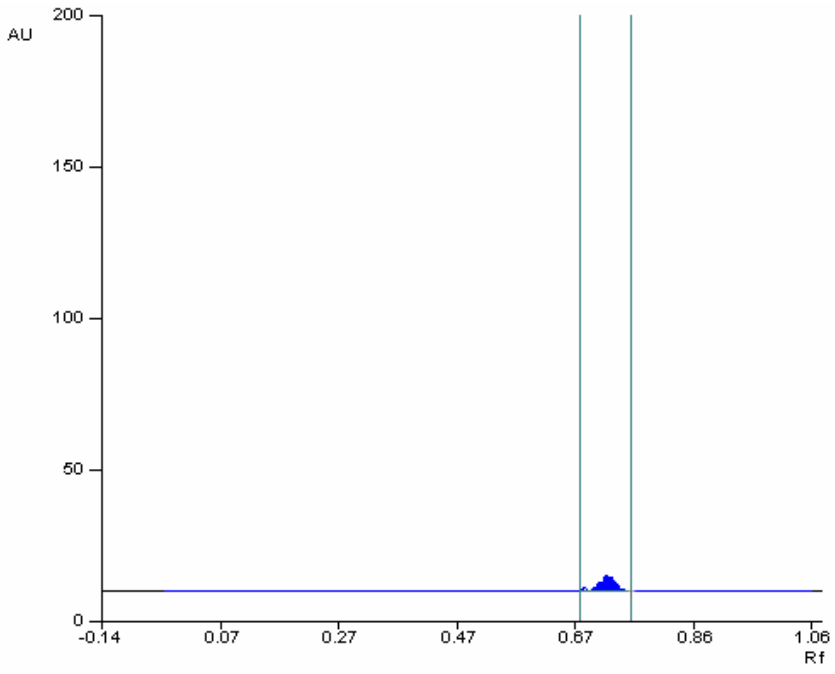
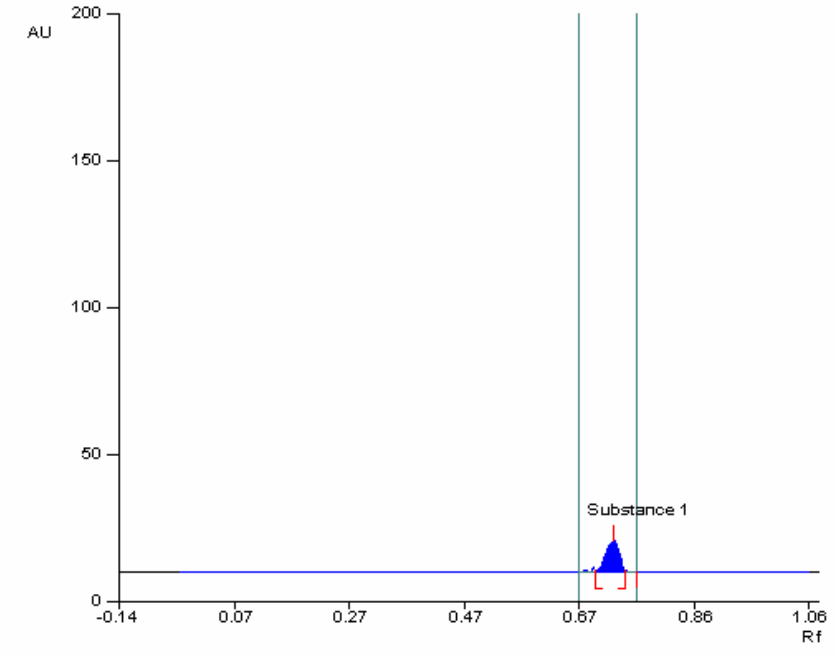


Fig. 32: LOQ



INTRODUCTION TO HPLC[18-22]

High performance liquid chromatography is a convenient separation technique used for wide types of samples, with exceptional resolving power, speed and nano molecular detection levels. This technique is based on the same modes of separation as that of classical chromatography i.e. adsorption, partition ion exchange and gel permeation, but it differs from column chromatography in the fact that the mobile phase is passed through the packed column under high pressure.

According to the phases involved, HPLC can be classified into several types which are as follows:

a) Partition chromatography
   - Normal phase chromatography
   - Reverse phase chromatography

b) Liquid- solid chromatography or adsorption HPLC

c) Liquid- liquid chromatography or partition HPLC

d) Ion exchange chromatography

e) Size exclusion or gel permeation

f) Ion- pair HPLC

g) Affinity HPLC

Parameters Used in Chromatographic Characterization

Retention

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of this are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (k), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$k_A = \frac{V_A - V_o}{V_o} = \frac{t_A - t_o}{t_o}$$

where,

V_A = Elution volume of A

V_0 = Elution volume of a non retained compound (void volume)

Resolution

The distance between any two adjacent peaks in a multi peak chromatogram is referred to as Resolution 'R_S' and is calculated as

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2}$$

where,

t_1 and t_2 are the retention times for the latest and the earliest eluting peak and W_1 and W_2, are the peak width at baseline.

$R \geq 1$ = Components completely separated

$R \leq 1$ = Components overlap

Capacity Factor (**k´**)

The retention of the analyte expressed as the number of void volumes of the system, needed for the peak to elute is called the capacity factor. The expression for k´ is

$$k' = \frac{t_r - t_0}{t_0}$$

where,

t_r = retention time

t_0 = void volume

Theoretical Plates (N)

The number of theoretical plates generated on a column is a measure of its performance. The definition of N is

$$N = 5.54\left(\frac{t_r}{t_{W1/2}}\right)$$

where,

t_r = retention time

$t_{w\ 1/2}$ = is the peak width at half height

'N' may also be calculated from the width along the baseline of the peak. This is accomplished by extending tangents from the two peak inflection points through the baseline.

Separation factor (∝)

This parameter is used to quantify the separation between adjacent peaks. Ideally, the peaks should not overlap, that is they should be

baseline-resolved. This condition is met for peaks of similar size when α **>** 1.15. The separation factor is calculated as follows

$$\alpha = \frac{K'_2}{K'_1}$$

where,

the subscripts refer to the order of elution.

$\propto$ is always ≥ 1

Asymmetry

The asymmetry is a tool for quickly determining how much if any, of an eluting peak profile deviates in shape from a normal distribution. The subscript 'x' refers to the percentage of peak height at which the asymmetry is determined.

Eg: A^{10} (determined at 10% peak height)

Peak asymmetry is given as:

$$A_x = b/a$$

where,

b = the distance between the perpendicular connecting the baseline to peak maximum and the latest eluting portion of the curve

a = the distance between the perpendicular connecting the baseline to the peak maximum and the earliest eluting portion of the curve.

QUANTITATIVE ANALYSIS

Quantification involves the measurement of peak height or peak area. To determine the concentration of a compound, the peak area or height is plotted Vs the concentration of the substance. For well resolved peaks, both peak height and area are proportional to the concentration. Three different calibration methods used in quantitative analysis is external standard, internal standard and the standard addition method.

RETENTION IN RP-HPLC

The reverse phase chromatography is based upon its polarity and experimental conditions such as mobile phase, column and temperature.

Mobile Phase effects

Retention (compound K value) can be preferably adjusted by changing mobile phase composition or solvent strength. In RPC, retention is less for stronger, less polar mobile phase. Solvent strength depends on the choice of organic solvent and its concentration in the mobile phase. A retention range of $0.5 < k < 20$ are allowable for samples to be separated using isocratic condition but $1 < k < 10$ is generally preferred.

a) Choice of organic phase (%B)

A mobile phase of 100% ACN is a stronger polar solvent, which may result in ($k < 0.2$), so a weaker mobile phase is required to retain the compound. This can be attained by decreasing the percentage of ACN

which in turn increases the retention time. When organic phase is decreased by 10% the k value increases 3 times approximately.

b) Mobile-phase strength

Mobile phase strength in RPC depends upon both % B and the type of organic solvent. RPC solvent strength varies as water (weakest) < methanol < acetonitrile < ethanol < tetrahydrofuran < propanol < methylenechloride (strongest). Solvent strength increases as solvent polarity decreases.

Column and Temperature effect

An increase in temperature by $1^{o}C$ will usually decrease values of k by 2% for non-ionic compounds, but it is not mostly used in RPC. For very hydrophobic samples it can be useful to operate at higher temperatures with a very strong mobile phase and very weak column.

SELECTIVITY IN RP-HPLC

Once overall sample retention is adjusted ($0.5 < k < 20$), it is necessary to change the band spacing or selectivity (α) of different bands. Three main variables used in RPC to change selectivity for neutral samples are mobile phase, column type and temperature. A small change in 'α' is adequate for separating many samples.

a) Solvent- strength selectivity

The best sample resolution will occur for a %B value where both pairs have the same resolution peak spacing can be explored while %B is varied for optimum sample retention ($0.5 < k < 20$). The use of solvent strength selectivity is limited mainly by the retention range of the sample.

b) Solvent Type selectivity

A change in organic solvent type is often used to change peak spacing and improve resolution. The selection of RPC solvents for this purpose is guided by solvent properties that are believed to affect selectivity, acidity, basicity and diploarity.

c) Column Type selectivity

A change in column type can produce useful changes in selectivity and overall sample retention. Retention is greater on the stronger C_8 and phenyl column Vs the weaker cyano column.

d) Temperature Selectivity

Values of k decreases at higher temperature for the RPC separation of neutral compounds. This is less effective for non-ionic compounds.

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TOLTERODINE TARTARATE FROM TABLET DOSAGE FORM BY RP-HPLC.

METHOD DEVELOPMENT

Shimadzu HPLC System – Class LC 10VP system with Photodiode array detector was used for the study.

1. **Selection of Stationary Phase**

Since Tolterodine tartarate is polar in nature, RP-HPLC method with C_{18} column was used for the development of the method.

2. **Selection of the Solvent**

The drug was dissolved in the mobile phase and suitably diluted and injected into the system.

3. **Selection of Detection Wavelength**

Good analytical results will be obtained only by careful selection of the wavelength used for detection. This choice requires knowledge of the UV spectra of the individual sample components. A UV spectrum of the drug was recorded and 282 nm was selected as the detection wavelength. (Fig. 33)

4. **Selection of the mobile phase**

Based on the solubility, different mobile phases were tried. (Fig. 34-36)

i) **Water : methanol** (50:50 %v/v) resulted in a **broad peak** with a R_t of 4.11

ii) **Water (pH 3.4): methanol** (50:50 %v/v) resulted in a **broad peak** with a R_t of 21.6

iii) **Water (pH 4.8): methanol** (10:90 %v/v) resulted in a **split peak** with a R_t of 1.82

iv) **Water (pH 8.7): methanol** (80:20 %v/v) resulted in a **split peak** with a R_t of 1.56

v) **Water: acetonitrile** (50:50 %v/v) resulted in a **broad peak** with a R_t of 11.45

vi) The drug **did not elute** in **Potassium dihydrogen phosphate buffer (pH 9.5):methanol** (30:70 %v/v)

vii) 50 mM Sodium acetate buffer (pH 9.45): methanol (10:90 %v/v) resulted in a good symmetrical peak with a R_t of 2.43

5. **Optimization of the Separation Conditions**

a) Effect of Ionic strength

Different ionic strengths of the Sodium acetate buffer such as 10 mM, 25 mM, 50 mM and 100 mM were tried. The pH was adjusted to 9.45 with 1% Triethylamine and mixed with methanol in the ratio 10:90 %v/v and their effect on the peak shape was observed. (Fig. 37-40)

i) 10 mM Sodium acetate buffer: methanol resulted in tailing of the drug peak.

ii) 25 mM Sodium acetate buffer: methanol resulted in tailing of the drug peak.

iii) **50 mM Sodium acetate buffer: methanol resulted in a good symmetrical peak.**

iv) 100 mM Sodium acetate buffer: methanol resulted in tailing of the drug peak.

b) Effect of pH

Different pH solutions of the 50 mM Sodium acetate buffer such as 4.3, 4.5, 8.5 and 9.45 were prepared and pH adjusted with 1% triethylamine. A mobile phase consisting of 50 mM Sodium acetate buffer and methanol in the ratio 10:90 %v/v was used and their effect on the peak shape was observed. (Fig. 41-44)

i) At **pH 4.3**, the drug peak resulted in giving a **peak shoulder**.

ii) At **pH 5**, the drug peak resulted in giving a **peak shoulder**.

iii) At **pH 8.5**, **peak tailing** was observed.

iv) At pH 9.45, a good symmetrical peak was observed.

c) Effect of Ratio

50 mM Sodium acetate buffer was prepared and the pH was adjusted to 9.45 with 1% triethylamine. The mobile phase consisting of 50 mM Sodium acetate buffer and methanol in various ratios such as 15:85, 5:95, and 10:90 %v/v were prepared and the chromatograms were recorded. (Fig. 45-47)

i) In the ratio **5:95 %v/v, split peak** was observed.

ii) **In the ratio 10: 90 %v/v, a good symmetrical peak was observed.**

iii) In the ratio **15:85 %v/v, tailing** of the drug peak was observed.

d) Effect of Flow rate

Keeping the ionic strength, pH of the buffer and the ratio of the mobile phase fixed, the chromatograms were recorded at different flow rates such as 0.8, 1.0 and 1.2 ml/min. (Fig. 48-50)

i) At **0.8 ml/min**, the retention time got shifted to **3.1min**.

ii) At **1.2 ml/min**, the retention time got shifted to **1.9 min**.

iii) At 1.0 ml/min, a good symmetrical peak was observed with a R_t of 2.43 min.

7. Fixed Chromatographic Conditions

Stationary Phase	: Gemini, Phenomenex C_{18} (150 x 4.6 mm, 5 µ)
Mobile Phase	: 50 mM Sodium acetate Buffer: Methanol
Ratio	: 10: 90 %v/v
Flow rate	: 1.0 ml/min
Detection Wavelength	: 282 nm

8. Preparation of the Stock Solution

10 mg of Tolterodine tartarate was weighed accurately and transferred into a 10 ml standard flask, dissolved in the mobile phase to

get a concentration of 1000 µg/ml. This stock solution was suitably diluted with the mobile phase to get a concentration of 100 µg/ml.

9. Preparation of Standard solution

From the Stock solution 0.2, 0.4, 0.6, 0.8,1.0 ml were transferred into a series of 10 ml standard flasks and the volume was made upto 10 ml with the mobile phase to get concentrations ranging from 2 to 10 µg/ml. Chromatograms of these solutions were obtained by injecting 20 µl of each standard solution into the system and the peak areas were recorded. The peak areas were plotted against the corresponding concentrations (Table : 25), calibration graph was constructed and the slope, intercept and correlation coefficient was determined. The standard solutions were found to be linear in the range of 2 to 10 µg/ml with a correlation coefficient of 0.9991. (Fig. 51-56)

Table : 25 Peak area values of Tolterodine tartarate

Concentration (µg/ml)	Peak Area
2	**13455**
4	**28214**
6	**40675**
8	**52915**
10	**64944**

10. Analysis of the Formulation:

20 tablets (Roliten, Ranbaxy Laboratories Limited, India) each containing 2 mg of Tolterodine tartarate were weighed and the average weight was calculated. A quantity equivalent to 10 mg of Tolterodine tartarate was transferred into a 10 ml standard flask and extracted with the mobile phase by shaking for 30 minutes. The solution was filtered through a Whatman filter paper and suitably diluted with the mobile phase to get a concentration of 100 µg/ml. From this, suitable aliquots of concentration 6 and 8 µg/ml were prepared. A chromatogram of these solutions were obtained by injecting 20 µl of each sample solutions into the system (Fig. 57)The peak areas were recorded and the amount of Tolterodine tartarate in the formulation was determined.(Table : 26)

Table : 26 Analysis of Formulation

Drug	Amount (mg/tablet)		%Label claim*± S.D
	Labeled	Found	
Tolterodine tartarate	2	1.99	99.89 ± 1.33

*Mean of six determinations

METHOD VALIDATION

The developed method was validated as per ICH guidelines and the standard deviation and relative standard deviation were calculated using the formula:

$$\textbf{S.D} = \sqrt{\frac{\sum\left(x-\bar{x}\right)^2}{n-1}}$$

$$\textbf{\% RSD} = \frac{100\ x\ S.D.}{\bar{x}}$$

Where, S.D. = standard deviation

R.S.D = relative standard deviation

1. Specificity

There were no additional peaks found with the alteration in the experimental conditions such as pH, flow rate, ratio etc., which revealed that the developed method was specific for the drug.

2. Linearity and Range

From the Stock solution, 0.2 to 1.0 ml were transferred into a series of 10 ml standard flasks and the solutions were made upto volume with the mobile phase to get concentrations ranging from 2 to 10 µg/ml .Calibration graph was prepared by plotting peak area Vs concentration .Tolterodine tartarate showed good linear relationship in the concentration range 2-10

µg/ml and the slope, intercept and correlation coefficient was found to be 6383.95, 1736.9 and 0.9991 respectively.

3. Precision

Precision of the method was determined by:

a. Intra-day precision

b. Inter-day precision

c. Repeatability of Injection

a. Intra-day precision

Intra-day precision was determined by injecting three solutions of the same concentration (4 µg/ml) for three times on the same day and the response for each injection was measured .Peak areas were noted and %RSD was calculated. (Table : 27)

Table : 27 Intra-day precision

Drug	Concentration (µg/ml)	Peak Area	%RSD*
Tolterodine tartarate	4	28507	1.47
		28040	
		28846	

* Mean of three determinations

b. Inter-day precision

Inter-day precision was determined by injecting a concentration of the standard solution (4 µg/ml) for three days and %RSD was calculated. (Table : 28)

Table : 28 Inter-day precision

Drug	Concentration (µg/ml)	Days	Peak Area	%RSD*
Tolterodine tartarate	4	I	28507	1.47
			28040	
			28846	
		II	28475	0.52
			28496	
			28366	
		III	28183	0.94
			28033	
			28532	

*Mean of three determinations

c. Repeatability of Injection

The standard solution of Tolterodine tartarate of concentration 4 µg/ml was injected three times and the response for each injection was recorded and the %RSD was calculated. (Table : 29)

Table : 29 Repeatability of Injection

Drug	Concentration (µg/ml)	Peak Area	%RSD*
Tolterodine tartarate	4	28428	0.63
		28735	
		28465	

*Mean of three determinations

4. Accuracy

The accuracy of the developed method was determined by conducting the recovery studies at 100% level .To the pre-analyzed formulation, standard drug of Tolterodine tartarate was added at 100% concentration level .The recovery procedure was repeated three times and %recovery was calculated. (Table : 30)

Table : 30 Recovery Studies

Drug	%Recovery*	%RSD
Tolterodine Tartarate	103.37	0.52

* Mean of six determinations

5. Limit of Detection and Limit of Quantification

LOD and LOQ of the developed method were determined by injecting low concentrations of the standard solution under optimized chromatographic conditions. LOD was found to be 10 ng/ml and LOQ was found to be 0.1 µg/ml. (Fig. 58, 59)

6. Stability

Stability of the analyte in stock solution was checked under room temperature and refrigeration conditions. The stored solution was found to be stable under room temperature for 24 hrs and under refrigeration conditions for 48 hrs. (Fig. 60, 61)

7. System suitability studies

System suitability studies were conducted as per USP specifications for the developed method and Column efficiency (N), Capacity factor (k´) and tailing factor were established. (Table : 31)

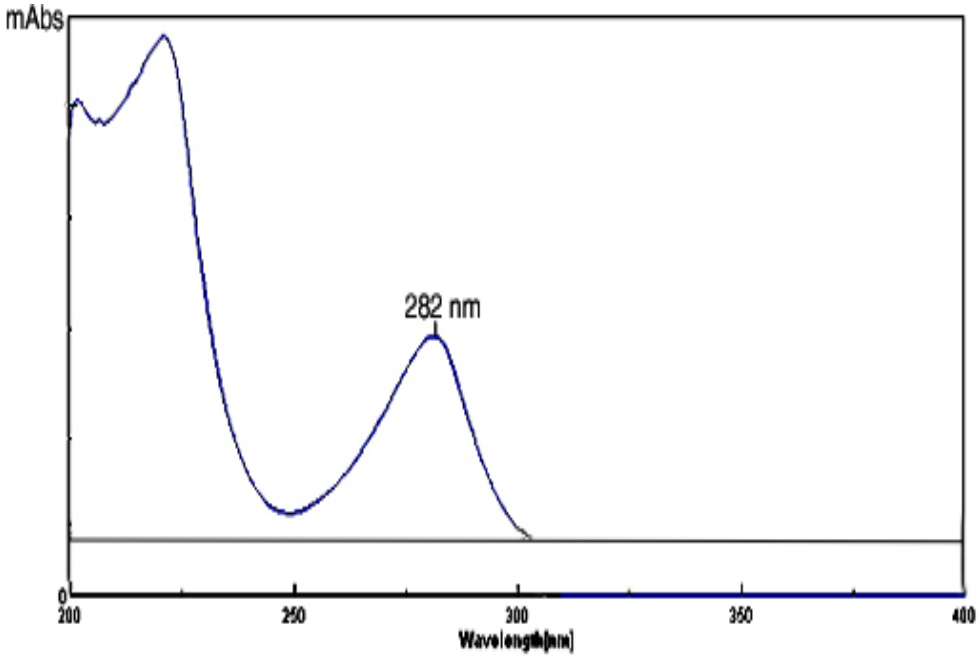
Table : 31 System suitability studies

Column efficiency (N)	**4560**
Tailing factor	**1.36**
Capacity factor (k´)	**1.21**

Results and Discussion

The results obtained by this method are precise and reproducible for the estimation of Tolterodine tartarate in its tablet dosage form. The percentage recovery was found to be 103% ± 0.52 with low level of S.D. which indicates the accuracy of the method. The content of Tolterodine tartarate in the tablet dosage form was found to be 1.99 mg/tab. Hence, the developed method can be used for the routine analysis of Tolterodine tartarate in the tablet dosage form.

Fig.33 : U.V. Spectra of Tolterodine tartarate in mobile phase



SELECTION OF MOBILE PHASE

Fig. 34: Water : Acetonitrile (50:50 %v/v)

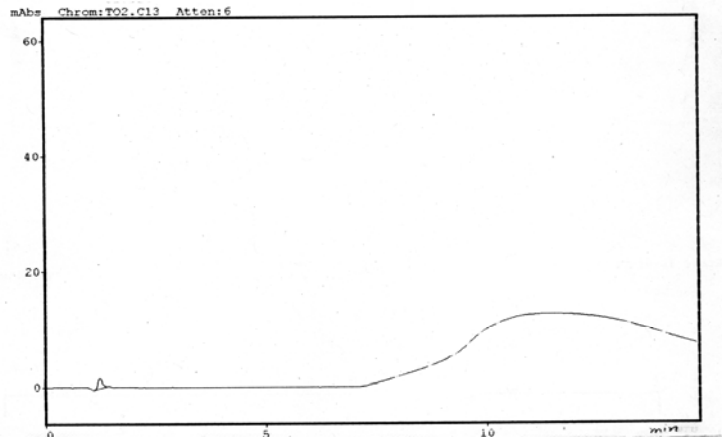


Fig. 35: Water : Methanol (50:50 % v/v)

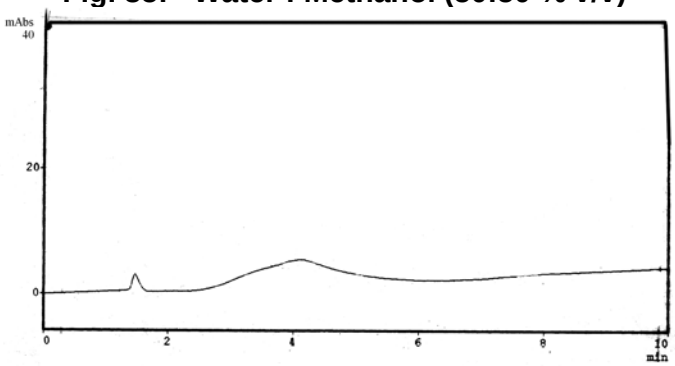
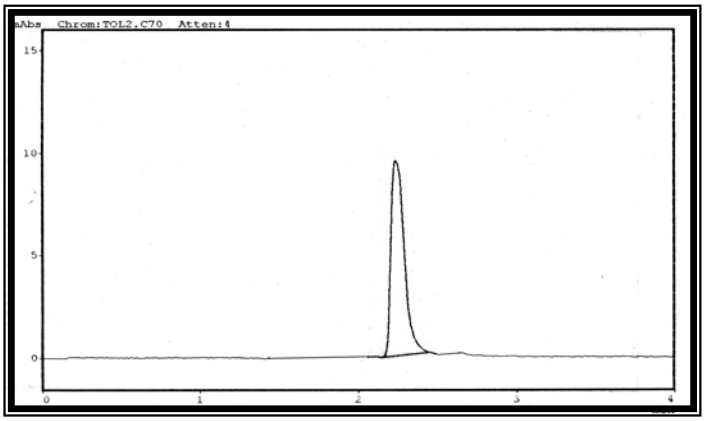


Fig. 36: 50 mM sodium acetate buffer (pH 9.45):methanol(10:90 %v/v)



EFFECT OF IONIC STRENGTH

Fig. 37: 10 mM Sodium Acetate buffer: methanol (10:90 %v/v)

Fig. 38: 25 mM Sodium Acetate buffer: methanol (10:90 %v/v)

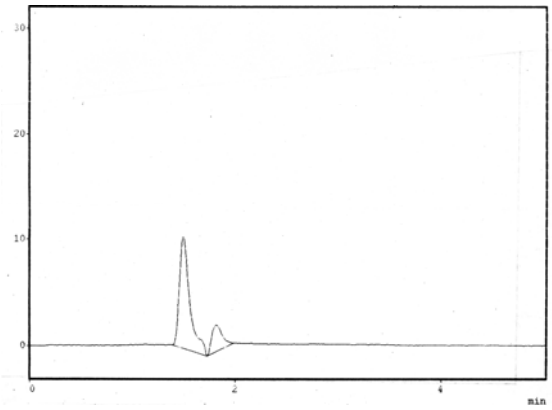


Fig. 39 : 50 mM Sodium Acetate buffer : methanol (10:90 %v/v)

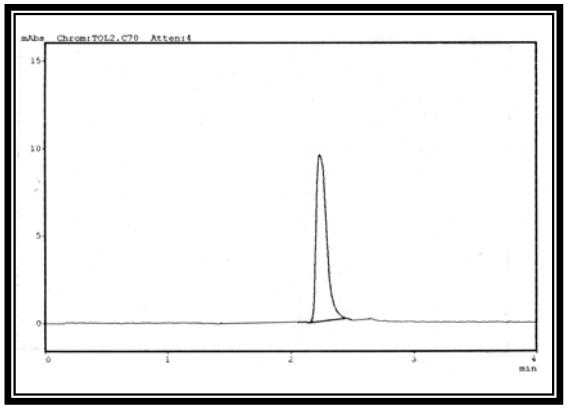
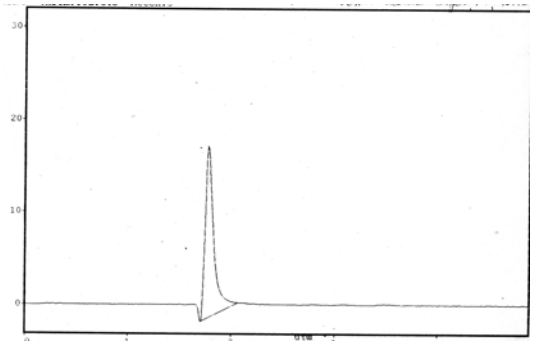


Fig. 40 : 100 mM Sodium Acetate buffer : methanol (10:90 %v/v)



EFFECT OF pH

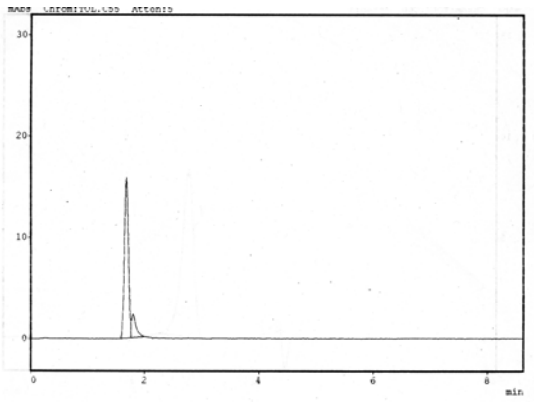


Fig. 41: pH 4.3

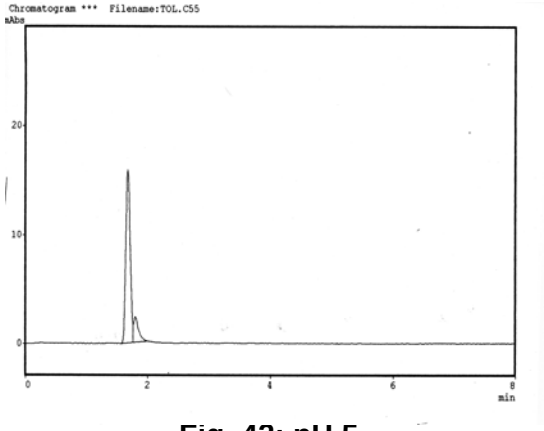


Fig. 42: pH 5

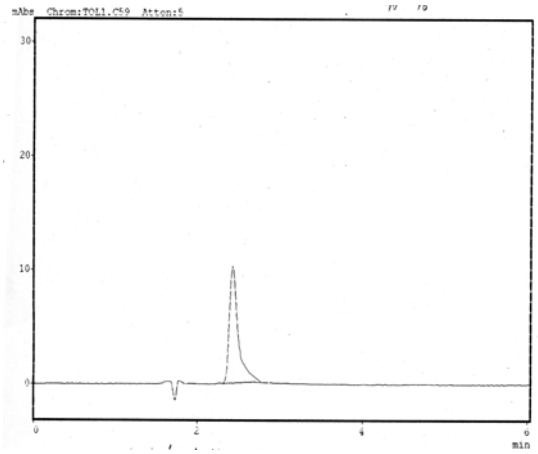


Fig. 43: pH 8.5

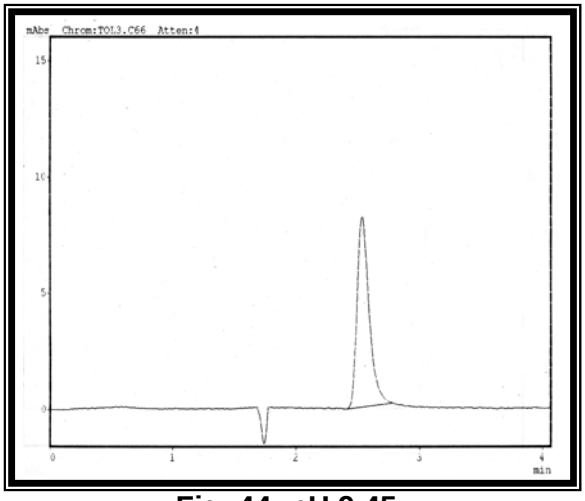


Fig. 44: pH 9.45

EFFECT OF RATIO

Fig. 45: 5:95 %v/v

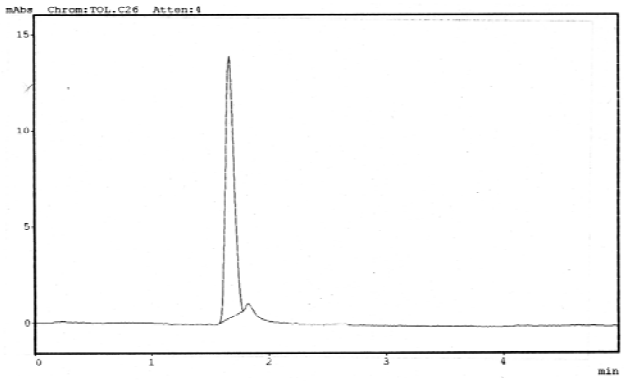


Fig. 46: 10:90 % v/v

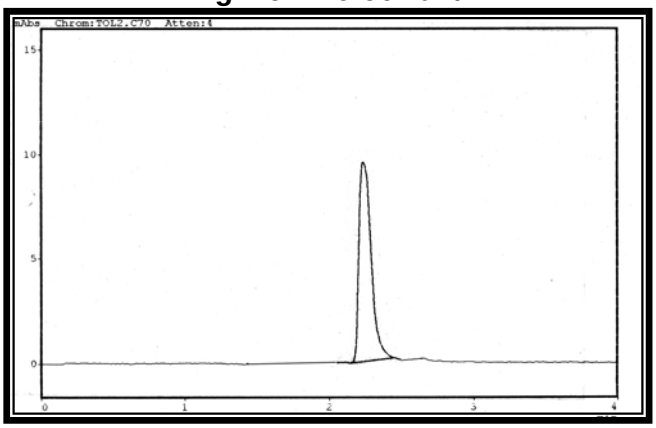
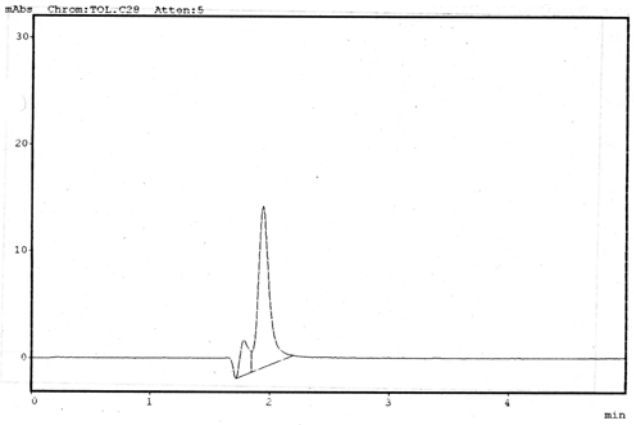


Fig. 47: 15:85 %v/v



EFFECT OF FLOW RATE

Fig. 48: 0.8 ml/ min

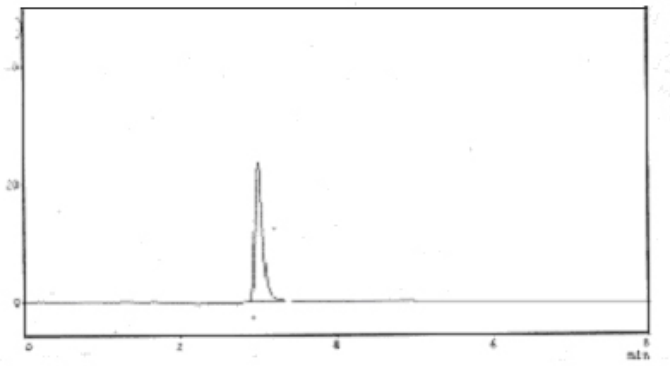


Fig. 49: 1.0 ml/ min

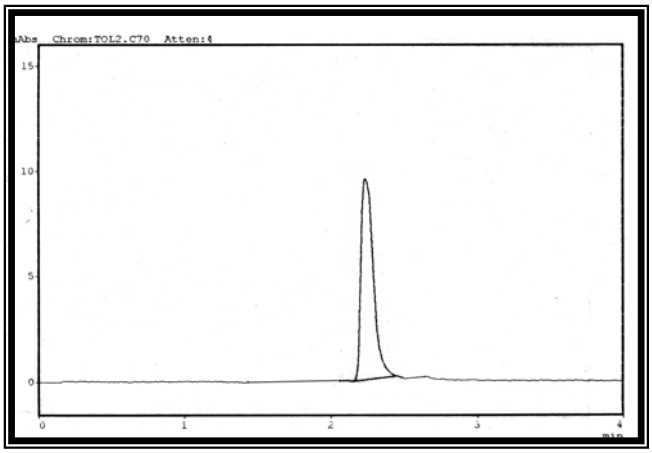
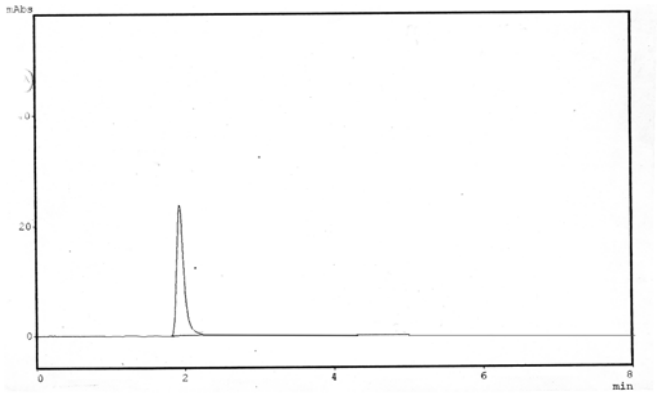


Fig. 50: 1.2 ml/min



CHROMATOGRAM OF STANDARDS

Fig. 51: 2μg/ml

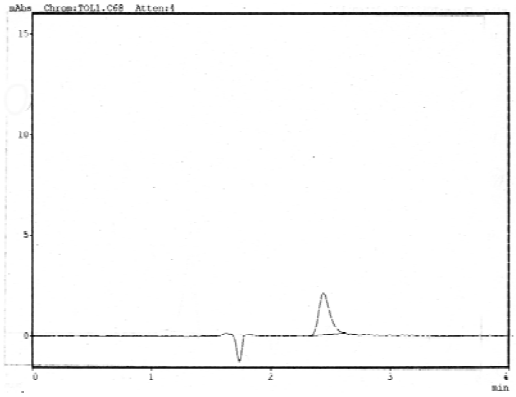


Fig. 52: 4 μg/ml

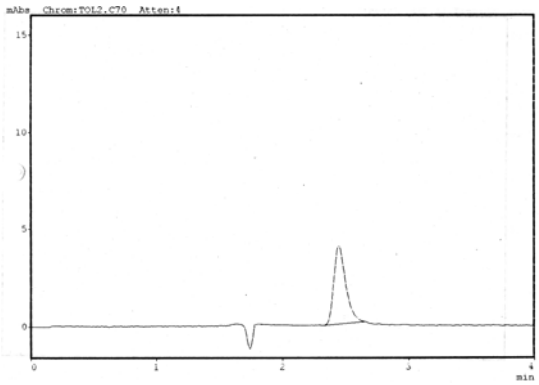


Fig. 53: 6 μg/ml

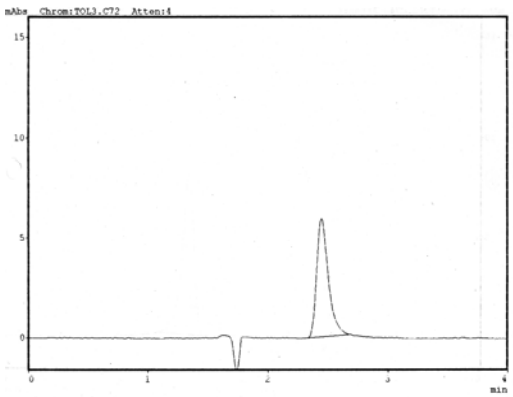


Fig. 54: 8 μg/ml

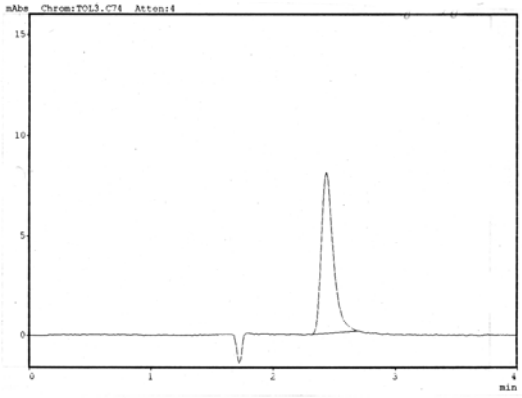


Fig. 55 : 10 μg/ml

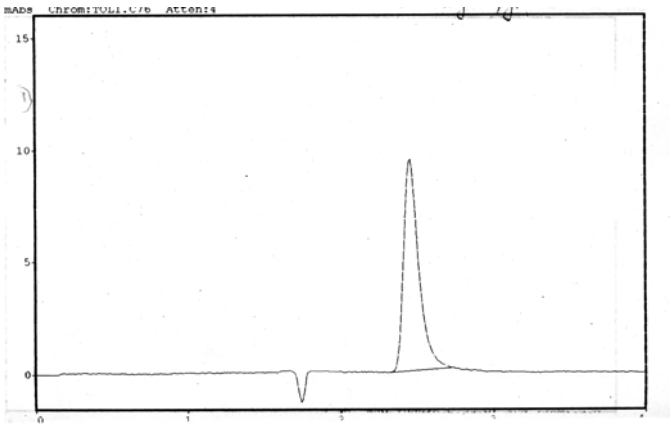


Fig.56 : Calibration graph of Tolterodine tartarate

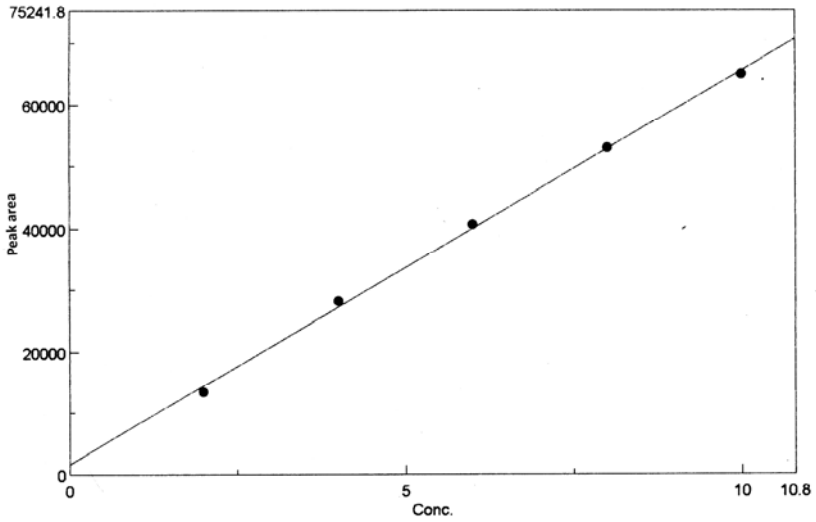


Fig. 57 : Chromatogram of formulation

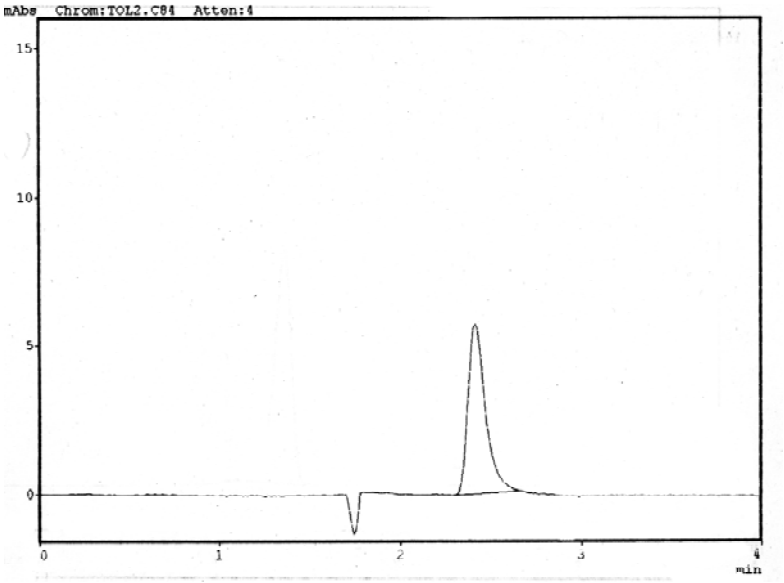


Fig. 58: LOD

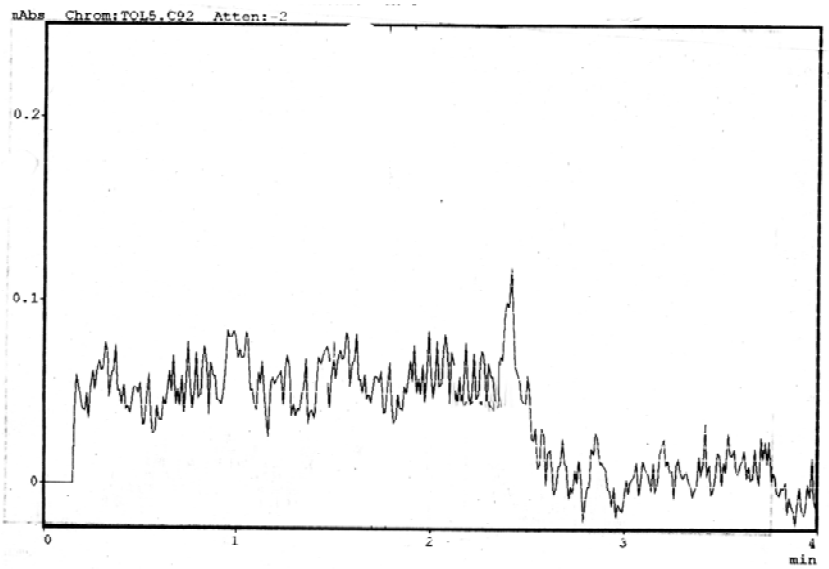


Fig. 59: LOQ

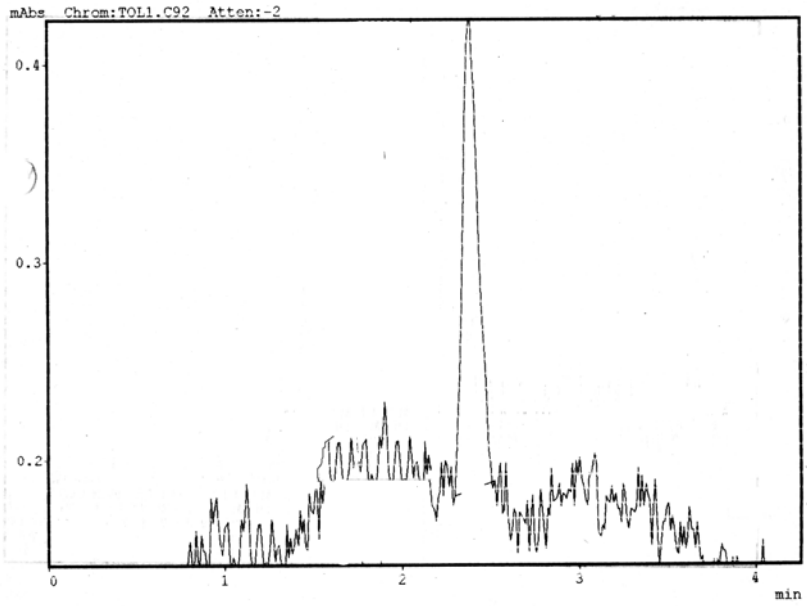


Fig . 60: Stability - Room temperature

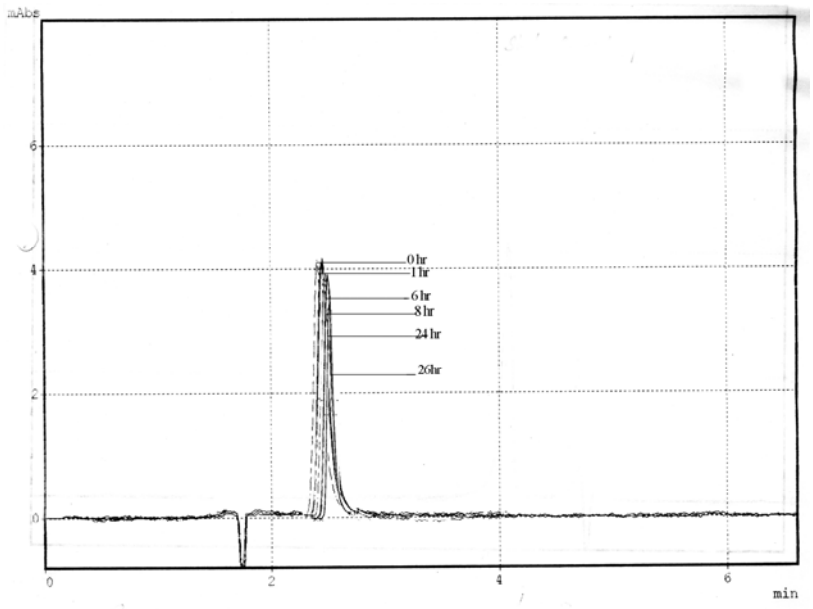
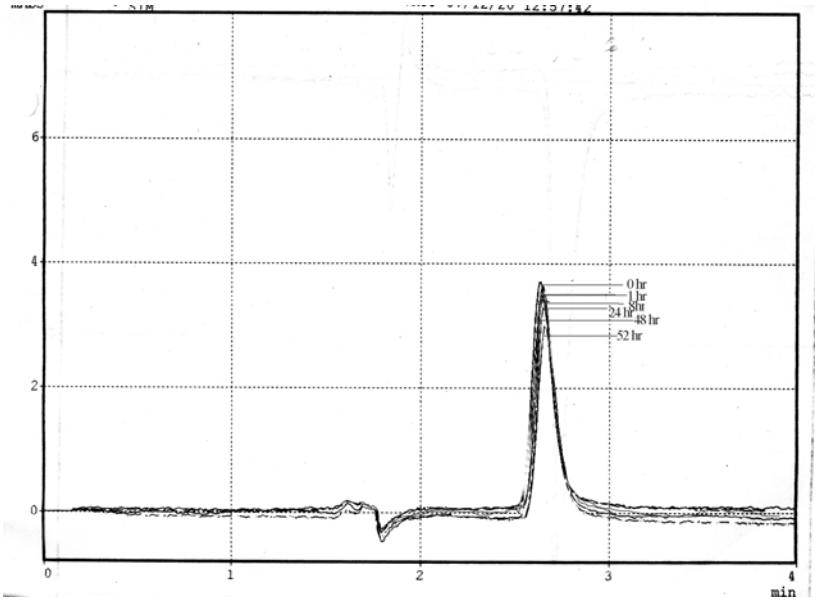


Fig. 61: Stability- refrigeration condition



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